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CONTENTS

A Cake Baking Method for Evaluating Wheat Varieties, *D. F. Sundberg, R. Hayashi, and M. A. Barmore* 1

The Heat Resistance of the Thermophilic Spores Present in Wheat and Wheat Products. *W. L. Jones, J. J. Jezeski, and W. F. Geddes* 12

Flavor of Bread. *J. C. Baker, H. K. Parker, and K. L. Fortmann* .. 22

The Effect of Fertilizer Application and Analysis on Certain Criteria of Malting Quality in Barley. *K. J. Frey and L. S. Robertson* 31

Determination of Calcium in Feeds by Flame Photometry. *Maxwell L. Cooley* 39

An Evaluation of Four Feed Sampling Devices. *P. R. Stout, J. W. Money, and M. P. Wineberg* 48

A New Color Method for Differentiating Bran and Germ Particles from Insect Fragments in Flour. *R. A. Larkin, M. M. MacMasters, and C. E. Rist* 54

Observations on a Sixty-Four Year Old Wheat Sample. *R. H. Harris and H. L. Walster* 58

Communication to the Editor 63

Book Reviews 66

Editorial Policy and Suggestions to Authors 69

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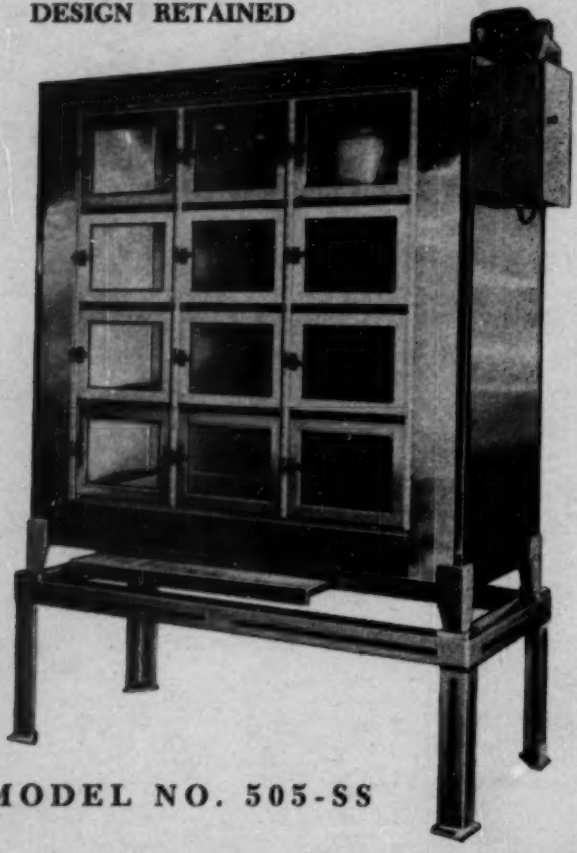

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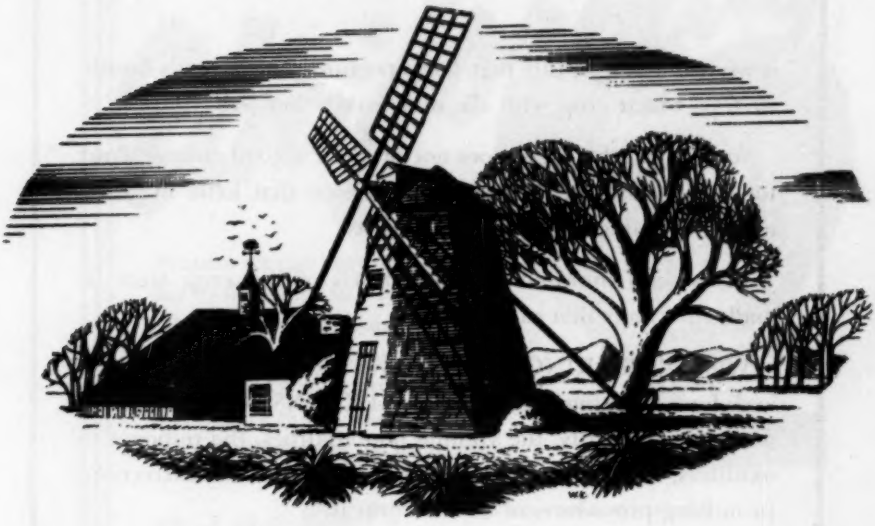

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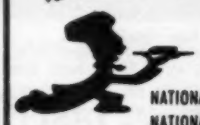

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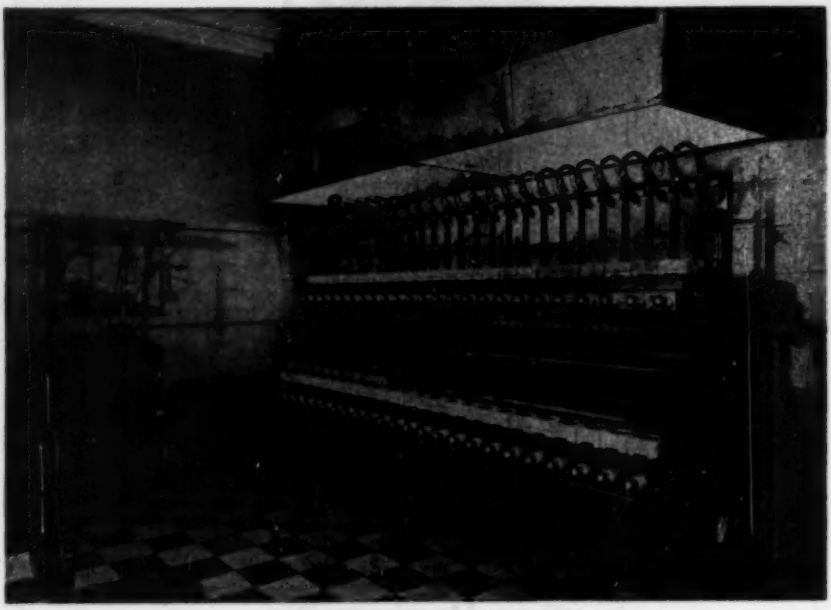

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CEREAL CHEMISTRY

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A CAKE BAKING METHOD FOR EVALUATING WHEAT VARIETIES[1]

D. F. SUNDBERG, R. HAYASHI, AND M. A. BARMORE[2]

ABSTRACT

Studies with single and two-stage methods of mixing cake batters employing commercially and experimentally milled flours showed that the two-stage method revealed greater differences between flours. In addition, results from variations in the amounts of some ingredients improved the sensitivity. Using the single-stage method, the variations in the quantity of ingredients produced differences in cake volume but did not increase the differentiation between flours with any particular amount. The two-stage mixing method appears to be much more sensitive to variations in flour since the differences in volume between the patent and the clear commercial cake flours varied from 42 to 136 ml. for the two-stage and from 32 to 63 ml. for the single-stage method, using variations in egg white and shortening. The comparison of the difference in the spread of volume, the similarity of texture scores and rank further emphasize the superiority of the two-stage method of mixing, the use of mulsifiers, and high sugar-shortening ratios for test baking.

Testing methods should, first, rank samples correctly and, second, be sensitive to real differences between samples. Layer cake baking methods previously tried in this laboratory have lacked sensitivity, especially in view of the replication errors. The dependence on cake volume is more desirable than internal characteristics as a basis for evaluating flours. The work described herein was undertaken to develop a method that would reflect, primarily in the cake volume, the difference in suitability of flours for making cakes. The paper consists of results of the effects of varying amounts of ingredients and mixing procedures on cake volume and internal characteristics.

Development of Improved Formulas

Two commercially milled cake flours were used in this study, a patent and a clear. These two flours were used to study the effect of

[1] Manuscript received March 3, 1952.

[2] Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agr. Res. Adm., USDA., Western Wheat Quality Laboratory, Department of Agricultural Chemistry, Pullman, Washington.

varying the quantities of ingredients and mixing procedures on the finished cake. A patent and a straight grade flour milled on the Buhler experimental mill from three commercially grown soft wheat varieties were also used. The three varieties were Baart, Elgin, and Hymar — Baart is generally considered very good for cake-baking purposes, Hymar considered only fair. The experimentally milled flours were bleached with chlorine containing 1% nitrosyl chloride to a pH of 5.0 to 5.2.

Moisture, ash, protein, absorption, and pH of each flour are given in Table I.

TABLE I

MOISTURE, ASH, PROTEIN, ABSORPTION, AND PH OF FLOURS USED

	Moisture	Ash[1]	Protein[1]	Abs.[1]	pH
	%	%	%	%	
Commercial cake patent	13.1	0.30	7.9	56	5.12
Commercial cake clears	12.5	0.48	8.2	64	5.40
Baart cake patent	13.1	0.31	7.2	57	5.20
Elgin cake patent	13.1	0.40	6.6	54	5.12
Hymar cake patent	13.6	0.37	6.4	56	5.08
Baart straight grade	13.1	0.38	7.7	59	5.09
Elgin straight grade	13.1	0.42	6.9	56	5.15
Hymar straight grade	13.6	0.42	7.0	57	5.18

[1] 14% moisture basis.

Single-Stage Method. After a preliminary study of the tentative formula of the 1947–48 A.A.C.C. cake committee, the following basic cake formula and procedure were employed in this study.

	% Based on Flour	*Weight, g.*
Shortening	45	72
Emulsifier	5[1]	3.6
Water	130[2]	208[2]
Flour	100	160
Sugar	135	216
Dry milk solids	12	19.2
Dried egg whites	10	16
Sodium chloride	2.5	4
Baking powder	5	8
Type mixer	3 speed Hobart Bench Machine, Model N-50	
Size of bowl	4 quart	
Attachment	Paddle	
Scale wt.	230 g. per 6 x 1½ in. round layer	
Baking temperature	375°F.	
Baking time	23 minutes (approximately)	

[1] % of weight of shortening.
[2] Dependent upon flour absorption.

Procedure. Place the shortening, emulsifier, and water in the bowl. Blend the dry ingredients; flour, sugar, dry milk solids, etc., and add to the above. Mix for ½ minute at low speed, 1½ minutes at high speed and ½ minute at low speed. Scrape the batter down after each change of speed. All cakes were removed from the pans the same day they were baked. The following day the cake volumes were determined by seed displacement and the internal characteristics judged.

Two-Stage Method. The second method of mixing differed from the first in that the sugar, shortening, and emulsifier were creamed before they were mixed with the other ingredients. The creaming procedure was as follows: 1134 g. sugar, 378 g. shortening, and 18.9 g. emulsifier were mixed for ½ minute at low speed, 2½ minutes at medium speed, and 4 minutes at high speed. The creamed mass was scraped down between each change of speed and again after mixing 2 minutes at high speed. Portions of the creamed mass (291.6 g. for 5% emulsifier) equivalent to 45% shortening and 135% sugar were weighed out and mixed with the other ingredients as before. The dried egg white content was also increased from 10 to 12%.

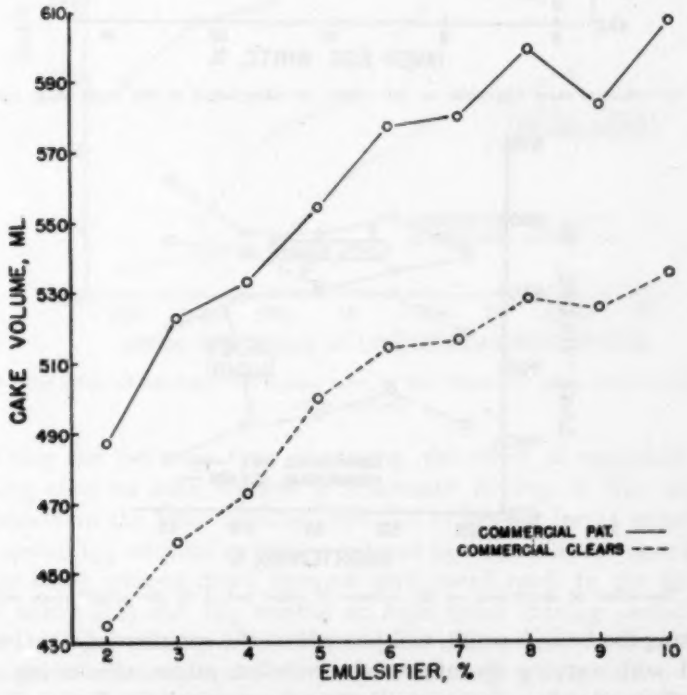

Fig. 1. The effect of emulsifier on the volume of cakes baked by the single stage method.

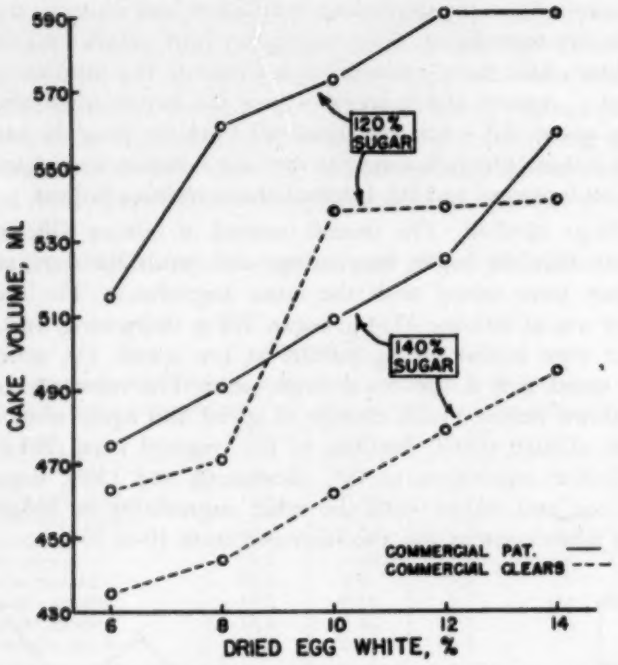

Fig. 2. The effect of dried egg white on the volume of cakes baked by the single stage method.

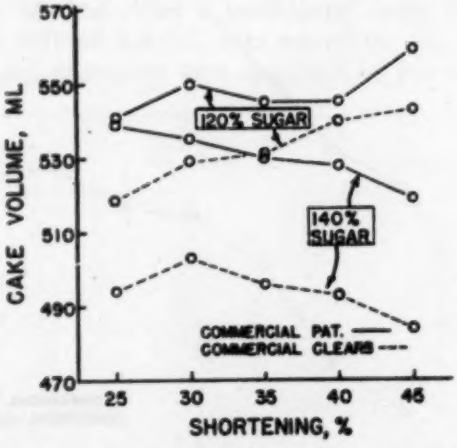

Fig. 3. The effect of shortening on the volume of cakes baked by the single stage method.

Using the basic formula and procedure, the commercial flours were baked with varying quantities of emulsifier, sugar, shortening, etc. The effects on cake volume are illustrated graphically in Figs. 1, 2, and

3. Increasing the emulsifier (Fig. 1) and dried egg white (Fig. 2) gave larger volumes but did not increase the differentiation between the two flours. Increasing the shortening gave increased cake volume with 120% sugar in the formula but decreased it with 140% sugar. Greater differences in cake volume at the higher sugar level are shown in Fig. 3.

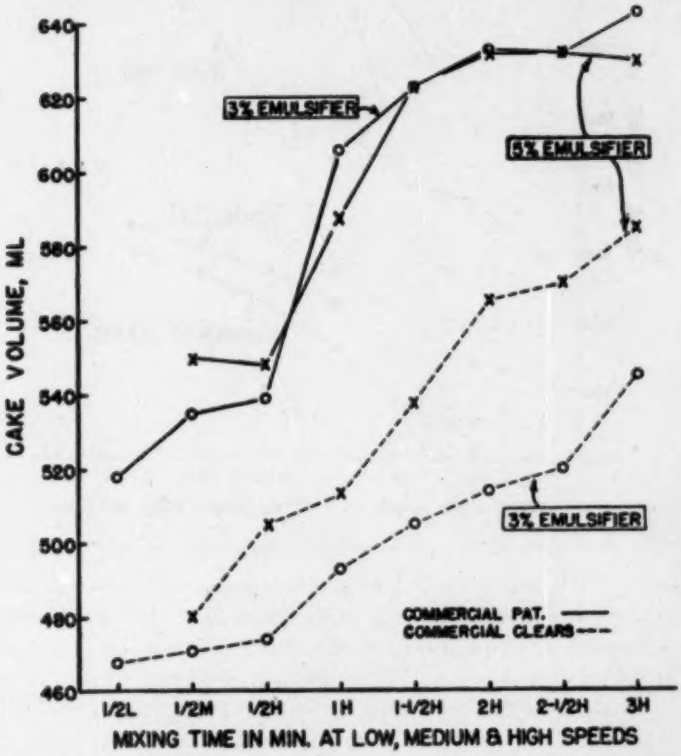

Fig. 4. The effect of emulsifier and mixing time on the volume of cakes baked by the two stage method.

Using the two-stage type of mixing, the effect of emulsifier and mixing time on cake volume is illustrated in Fig. 4. The mixing procedure in the basic formula consisted of mixing for ½ minute at low speed, 1½ minutes at high speed and ½ minute at low speed, the batter being scraped down between each speed used. In the mixing-time study only the 1½ minute at high speed mixing period was varied. Longer mixing time increased the differences in cake volume from the two flours. Figure 5 shows the effect of mixing time on cake volume of the three experimentally-milled flours and the two com-

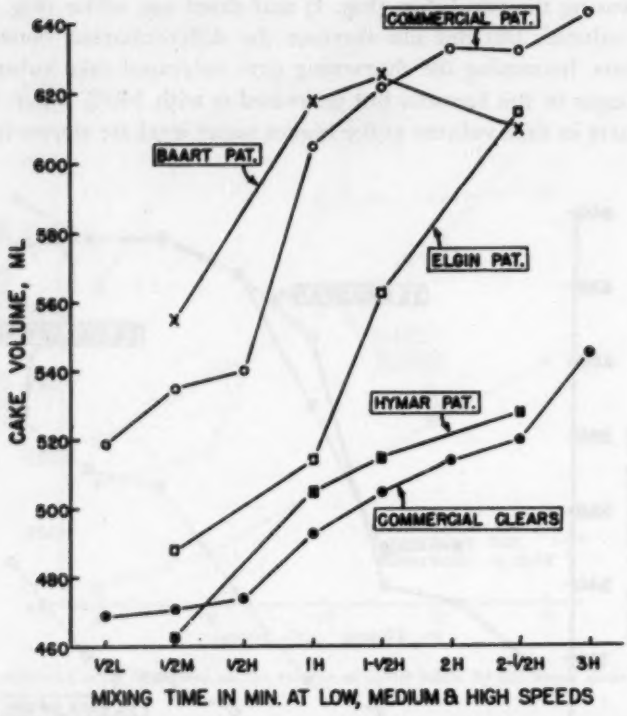

Fig. 5. The effect of mixing time on the volume of cakes baked by the two stage method.

mercial flours baked with 3% emulsifier using the two-stage mixing. The 1½ minute mixing time at high speed gave the best differentiation between flours and also seemed to rank them in the proper order.

The effect of increasing the percent of dried egg white (2) and of sugar on cake volume are shown in Figs. 6 and 7, respectively. Dried egg white at 12.5 or 13% and sugar at 135% gave the best differentiation and ranking of the flours. Only the commercially milled flours were baked with varying quantities of shortening. As illustrated in Fig. 8, the greatest difference in cake volume was shown with 45% shortening.

The most desirable formula and procedure, based on the data collected, seems to be:

	% Based on Flour	*Weight, g.*
Shortening	45	72
Emulsifier (monoglyceride)	3[1]	2.16
Sugar (Bakers special fine granulated)	135	216
Water	130	208
Flour	100	160

Dried egg whites	12.5	20
Dried milk solids	12	19.2
Sodium chloride	2.5	4
Baking powder (multiple action)	5	8

[1] % of weight of shortening.

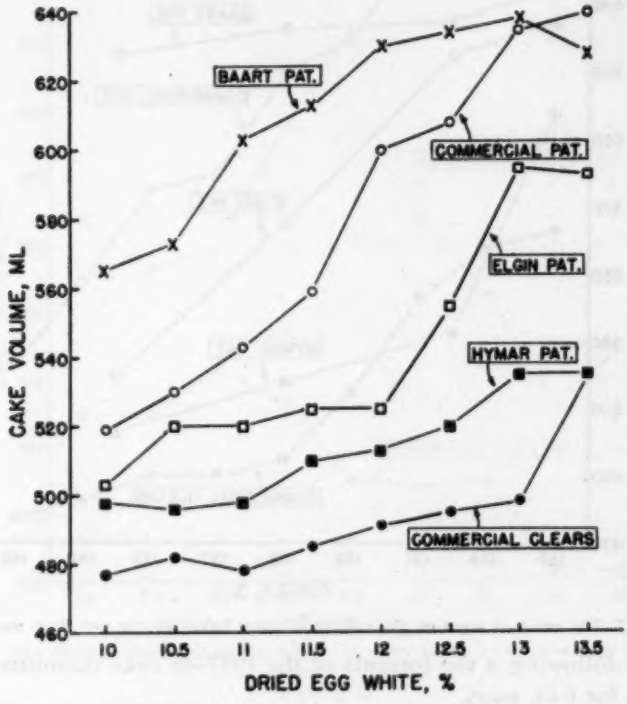

Fig. 6. The effect of dried egg white on the volume of cakes baked by the two stage method.

Procedure. Cream the shortening, emulsifier, and sugar at ½ minute low speed, 2½ minutes medium speed, and 4 minutes high speed. Add the water to the creamed mass. Blend the dry ingredients, flour, dried egg whites, etc., and add to the above creamed mass and water. Mix ½ minute at low speed, 1½ minutes at high speed, and ½ minute at low speed. Scale at 230 g. per 6 x 1½ in. round layer. Bake at 375°F. for 23 minutes.

Comparison of Three Cake Formulas

To illustrate the improvement that had been made in the cake formula and procedure, the above formula was compared with that of the 1947–48 AACC cake committee and that given in Cereal Laboratory Methods (1).

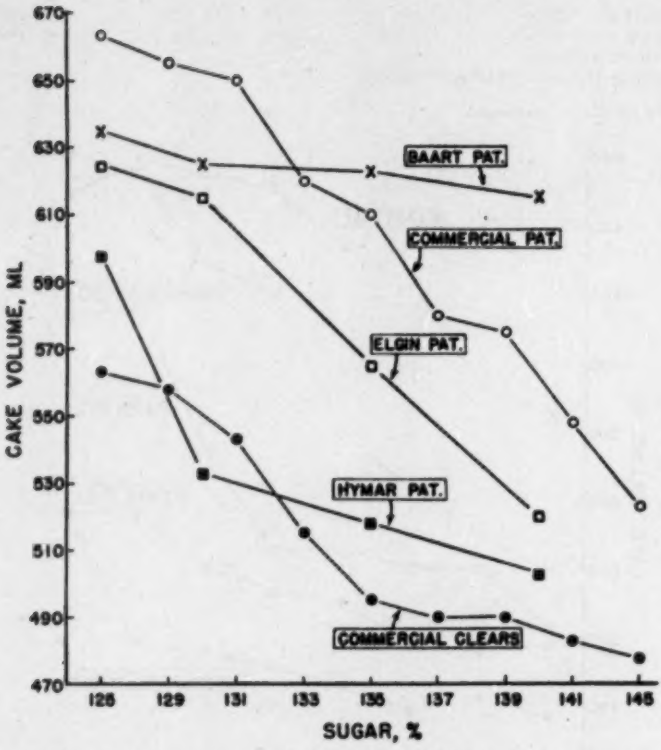

Fig. 7. The effect of sugar on the volume of cakes baked by the two stage method.

The following is the formula of the 1947–48 cake committee recalculated for 6-in. pans.

	% Based on Flour	*Volume, ml.*
Liquid skim milk	77.1	123.3
Egg whites	60.4	96.7
		Weight, g.
Shortening	45.8	73.3
Emulsifier	5[1]	3.67
Flour	100	160
Sugar	115	184
Sodium Chloride	2.9	4.64
Sodium acid Pyro phosphate	2.14	3.42
Sodium bicarbonate	1.48	2.38

[1] % of weight of shortening.

Procedure. Place the liquid skim milk, egg whites, shortening, and emulsifier in the bowl. Blend the dry ingredients—flour, sugar, salt, etc.

and add to the above. Mix ½ minute at low speed, 1½ minutes at high speed, and ½ minute at low speed. Scale at 230 g.per 6 x 1½ in. round layer. Bake for 23 minutes at 360°F.

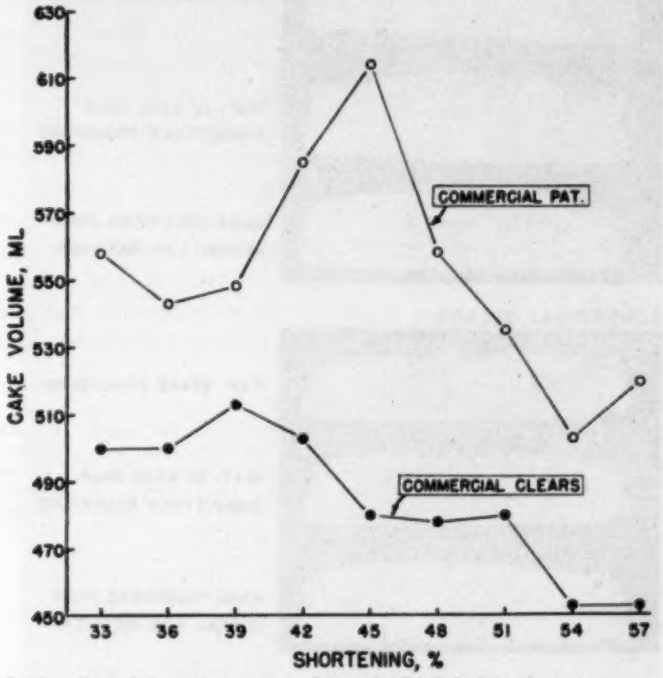

Fig. 8. The effect of shortening on the volume of cakes baked by the two stage method.

TABLE II

VOLUME AND RANK (GRAIN AND TEXTURE) OF THE CAKES BAKED BY THE THREE METHODS

	Two Stage Procedure		1947-48 AACC Cake Committee's Procedure		AACC Procedure from Cereal Lab. Methods	
	Cake Volume	Grain and Texture Rank	Cake Volume	Grain and Texture Rank	Cake Volume	Grain and Texture Rank
	ml.		*ml.*		*ml.*	
Baart patent	634	1	568	1	508	1
Commercial patent	616	2	544	3	500	7
Baart straight grade	601	2	558	2	521	2
Elgin patent	566	3	528	2	501	5
Elgin straight grade	576	4	528	5	505	3
Hymar patent	514	6	514	6	488	8
Hymar straight grade	509	7	505	7	474	6
Commercial clears	490	8	506	8	530	4
Standard deviation	8.6		10.1		13.9	

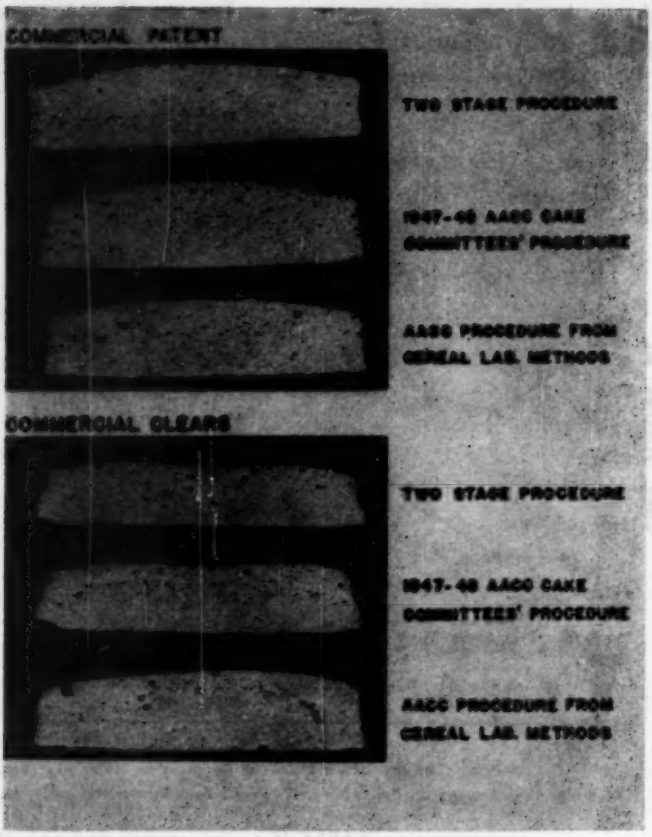

Fig. 9. The appearance of the internal structure of the cake baked by the three methods.

All eight flour samples were baked, using each of the three formulas. Cake volume and ranking of the cakes are presented in Table II. The appearance of the internal structure of each cake baked by the three methods is shown in Fig. 9.

Discussion

The two-stage mixing method was much more sensitive to variations in flour than the single-stage method. The differences between cakes from the commercially milled patent and the clear cake flours varied in volume from 42 to 136 ml. for the two-stage procedure and from 32 to 63 ml. for the single-stage method when using different percentages of egg white and shortening. With the two-stage method the proportions of sugar, shortening, dried egg white, and emulsifier

are critical in a cake formula when characterizing and differentiating between varieties. Proper amounts of these ingredients and proper mixing time are necessary to produce the maximum volume differences between samples. Greater differences between varieties in cake volume, ranking of varieties in accordance with commercial usage and the similarity of texture scores emphasize the superiority of the two-stage method of mixing, use of the proper amount of emulsifier, and high sugar-shortening ratios for test baking.

Both the commercially milled patent and clear cake flour gave larger cakes in every instance with added emulsifier, regardless of ingredients and mixing method used. Pyke and Johnson (2) reported the opposite effect, using different shortenings containing emulsifiers. They used a lower sugar-shortening ration, which would not explain the differences in results obtained. They also studied whole egg cakes which may partially explain the contradiction of results.

Literature Cited

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THE HEAT RESISTANCE OF THE THERMOPHILIC SPORES PRESENT IN WHEAT AND WHEAT PRODUCTS[1,2]

W. L. JONES[3], J. J. JEZESKI[4], AND W. F. GEDDES[5]

ABSTRACT

Thermophilic spore counts (aerobic and anaerobic) were made on 38 samples representing durum and common wheats and their products obtained from two large commercial mills. Most of the samples examined were relatively low in total thermophilic spore counts (from 2 to 74 spores per gram) and in the heat treatments required for sterilization. When these flours are used in canned goods, spoilage due to their use is unlikely, particularly if the heat treatment received is equal to or greater than an $F_s = 1.0$. No survival was observed when an $F_s = 2.0$ was used. The majority of the spores appear to be located on the surface of the wheat, in the aleurone layer and endosperm immediately beneath it and in the vicinity of the germ. Washing and tempering the grain as well as surface sterilization reduce the number of spores but do not eliminate them completely.

The problem of heat resistance of the microorganisms contained in raw foods has confronted the canning industry since its inception. In order to process a canned food ideally the heat treatments must be controlled carefully so that the final product is commercially sterile, and the palatability and food value are not impaired. While the prime consideration in heat processing is the elimination of health hazards from canned food, palatability and food value are limiting factors which influence the temperatures and times of heating which may be used. Thus, it becomes vital to know the resistance of the commonly occurring microorganisms to the heat treatments in general use in the canning industry. A commercial canning process involves two considerations from a bacteriological standpoint: (1) protection of the health of the consumer by destroying or preventing the growth of spores of *Clostridium botulinum*, (2) destruction, or preventing the growth of spoilage bacteria, usually of the thermophilic and mesophilic anaerobic spore-forming types.

Baumgartner (4) has presented a summary of the various classes of canned products and the type of microbial spoilage to which each is most susceptible. Mesophilic and thermophilic anaerobes are among

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[2] Paper No. 2835, Scientific Journal Series, Minnesota Agricultural Experiment Station. Taken from a thesis submitted to the Graduate Faculty of the University of Minnesota by W. L. Jones in partial fulfillment of the requirements for the degree of Master of Science.
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the most common organisms producing spoilage in canned foods which contain various cereal products. This is true particularly for semi-acid and low acid foods (pH 5.0 and higher), such as soups, gravy pastes, spaghetti and certain dog foods.

Wheat flours, although fairly widely used in the canning industry for such products, have not been studied thoroughly with regard to the heat resistance of the bacterial spores present, possibly because the total bacterial populations are, in general, low relative to many other common ingredients such as raw sugar, spices and soy flour.

Yesair and Cameron (14) examined 18 samples of corn flour, four of oatmeal flour, and 37 of wheat flour from various sources. Total thermophiles, flat sour spores, sulfide spoilage spores, and thermophilic anaerobes were determined and the samples judged for acceptability by the National Canners Association (NCA) sugar standards. Eleven of the 18 corn flour samples were below standard as were two of four oat flours. Only four of 37 wheat flour samples were unsatisfactory and these were only slightly so. They concluded that wheat flours were most suitable as ingredients of non-acid canned foods.

Recent studies by James and Smith (10) on the microflora of some Canadian flours have indicated somewhat greater thermophilic counts. However, a possible explanation is that their methods of inactivating vegetative cells were not so rigorous as the NCA methods used by Yesair and Cameron.

Clark and Tanner (8) compared a number of methods for the determination of thermophiles in starch and pointed out the importance of using the correct heat treatment for inactivating vegetative cells before making counts. Starch was found to be contaminated with thermophilic organisms possessing heat resistance similar to those found in sugar. Spoilage in spaghetti, canned corn, and dog food have been traced to starch contamination by Cameron and Williams (7).

A study of the heat resistance of thermophilic organisms is of prime importance and involves a determination of the time and temperature required to kill a suspension of these bacteria under a set of constant conditions if the data are to be applied to practical canning operations.

Bigelow and Esty (6) were among the first to suggest a standardized method for determining the heat resistance of bacteria. The suspensions of spores in appropriate media were placed in soft glass "thermal death-time tubes" which were then sealed and exposed to a series of temperatures. Individual tubes were removed from the bath at the end of selected time intervals, the tubes cooled and the contents

inoculated into nutrient broth. This "single tube" method had the disadvantage of producing occasional "skips"; that is, destruction of all organisms in a tube heated for a certain time, but survival of some organisms in a replicate tube heated for a longer period.

The multiple tube method of Esty and Williams (9) largely overcame the difficulty due to "skips". Instead of a single tube, 25 to 30 tubes, all containing aliquots of the same suspension, were exposed for each of at least four time intervals. The percentage of tubes showing survival of spores was then recorded. Upon plotting the logarithm of percent survival against the heating time in minutes, straight lines were obtained which could then be extrapolated to points of absolute destruction.

From a knowledge of the rates of destruction at a number of temperatures, a thermal death-time curve for an organism may be constructed. The application of laboratory-determined destruction rates and thermal death-time curves is complicated by the lag in heat penetration; as a result, the center of a container is not at the bath temperature during the entire exposure period. The heat penetration curve records the temperature at the center of the container during processing and cooling.

Bigelow, Bohart, Richardson, and Ball (5) were the first to devise a method for the computation of sterilization time from data provided by heat penetration and thermal death-time curves. Thermal death times were obtained at a series of temperatures in the processing range and the values were translated into lethal rates which were plotted against the logarithm of time.

Ball (1, 2) improved upon this method and introduced the terms "F" and "z" to characterize the thermal death-time curve. "F" is the number of minutes at a constant temperature of 250°F. required to destroy an organism. "z" characterizes the slope of the thermal death-time curve and actually represents "the increase in temperature (°F.) required to bring about a tenfold decrease in the thermal death time". Knowing the point "F" and the slope "z", the thermal death curve can be established. The value of "z" is given by the equation $\frac{T_2 - T_1}{\log t_2 - \log t_1}$ where T_2 and T_1 are temperatures, and t_2 and t_1 are the corresponding times. If $t_2 = 10\ t_1$ then $\log t_2 - \log t_1 = 1$, therefore $T_2 = T_1 = z$. F_0 has been used conventionally to designate the F value when $z = 18$.

The z value varies from 8°F. for certain lactobacilli to an average value of about 18°F. for spore formers of the type found in wheats and flours. Typical death-time curves having $z = 18$°F. are depicted in Fig. 1.

Thermal death determinations of short durations (exposure periods of ten minutes or less) require a correction for the heat penetration lag since the time required for the contents of the thermal death-time tube to reach bath temperature may comprise an appreciable part of the total exposure period. This fact has been recognized by Sognefest and Benjamin (12) but was neglected by many of the earlier workers. Appropriate corrections must be made so that the time of exposure to a varying temperature can be calculated to an equivalent time at 250°F., or at the constant bath temperature. For example, if from the heating curve, the average temperature for one minute is 227.3°F., reference to the z curve in Fig. 1 shows that the lethal effect is equivalent to 0.056 min. at 250°F. The correction factor is actually the difference between the time (minutes) to bring the tube to the temperature of the bath and the equivalent (minutes) in lethal effect at the constant bath temperature of the time, X. The time, Y, is obtained by a graphical or mathematical summation of the heating curve which may be obtained by the use of a fine wire thermocouple inserted in a thermal death-time tube submerged in the constant temperature bath. The whole heating and cooling curve (considered as being made up of a series of straight lines extending over intervals of one minute during which an average temperature is calculated) can be summed up and the total heat treatment stated as a time at a constant temperature. Ball (3) has discussed very thoroughly the role of the heat penetration curve in the calculation of thermal death-time values, and has explained the steps involved.

During growth and harvesting, wheat is exposed to heat-resistant thermophilic spores which are present in the soil and wheat flours must be considered a potential source of these microorganisms. The present study was undertaken to determine: (1) the extent to which spores of these microorganisms (both aerobic and anaerobic) may be encountered in wheats, flours and various mill streams; and (2) their resistance to heat under conditions comparable to canning practices. Some consideration was given to the effects of surface sterilization of the wheat on the spore count. The spore count of durum wheat harvested in one crop year and stored for almost twelve months was compared with that of newly harvested wheat.

Materials and Methods

Thirty-eight samples comprising both durum and common wheats and their products obtained from two large commercial mills were studied. Of these, five were obtained from a durum mill operating on a blend of 1948 crop wheats, namely, the mill mix before and after

washing and tempering, No. 1 semolina and first and second clear flours. A corresponding series of durum samples was obtained later from the same mill using 1949 crop wheat. Assays were also made of four samples of the durum wheat mixes which were surface sterilized with sodium hypochlorite. Twenty-four samples were obtained from a bread wheat mill operating on wheat mix made up of 50% hard spring, 1948 crop; 25% hard winter, 1948 crop; and 25% Southwest winter, 1949 crop. The samples included the wheat before and after tempering, several mill streams and different flour grades.

All samples were taken over a 5 minute interval directly from the millstreams and placed in sterilized 2 lb. Mason jars which were then stored at 5° to 10°C. The wheats and coarse mill fractions were ground in a micro hammer mill to pass through a 40-mesh sieve before examination. Between each sample the mill was cleaned thoroughly, rinsed with 95% ethanol and allowed to dry before use. Surface sterilization of the wheat was accomplished by shaking with 1% sodium hypochlorite solution for two minutes followed by a rinse of sterile 0.01*N* sodium thiosulfate to neutralize the excess chlorine. The treated samples were suction-dried in a Buchner funnel followed by a twelve hour exposure in a dessicator over sulfuric acid to reduce the moisture content to about 10%. The various samples were analyzed for moisture, protein and ash content according to the customary procedures.

Determination of Thermophilic Spore Counts (Aerobic and Anaerobic) For the determination of the various types of spores, methods based on those of James and Smith (10) were used. Ten grams of the ground sample were placed in a sterile stoppered cylinder with a small quantity of sterile quartz sand, 100 ml. water added and the cylinder shaken vigorously for 15 minutes. Before appreciable settling, 10 ml. of the suspension was pipetted into a flask containing 90 ml. of water immersed in a water bath at 90°C. After holding at this temperature for 25 minutes to destroy vegetative cells, the flask was cooled rapidly to room temperature and aliquots of the suspension pipetted into petri dishes which then were poured with dextrose-tryptone agar (Difco) containing brom-cresol-purple and incubated at 55°C. for 72 hours. Total counts represented the total aerobic thermophilic spores; the thermophilic flat sour spores were readily differentiated by the yellow zone surrounding the typical colonies. Anaerobic thermophilic spores were determined by adding aliquots of the final suspension to tubes of special liver broth and incubating at 55°C. for 5 days. Acid production and/or turbidity were accepted as evidence of the presence of at least one viable spore in the inoculum. All determinations were

carried out in triplicate or quadruplicate, three or four 10 g. samples being taken from each flour.

Thermal Death-Time Studies. Rather than determine precisely the equivalent F_0 required to destroy the spores present in the wheats and wheat products, a series of heat treatments equivalent to F_0 values commonly used in the canning industry were tested to determine which one would destroy the spores.

To determine the best bath temperature for carrying out the thermal death-time treatments (equivalent to F_0 of 0.5, 1.0, 1.5, and 2.0) consideration was given to the length of time which it was desired to keep the tubes submerged. These times should not be too short as the correction factor then becomes highly significant nor too long else small variations in the temperature of the bath become significant. A range of exposure times from 10 to 40 minutes for the F_0 treatments desired was found to occur at a temperature of 227.3°F. (108.5°C.).

Correction factors have been shown to vary little even with fairly wide differences in the composition of the paste (1). Except for the composition of the paste, the methods and conditions of heat treatment used in the present studies were essentially the same as those of Stumbo, Gross and Vinton (13). Accordingly, the correction factor of 1.98 minutes for 227°F. was taken from their data.

The exact times during which the tubes must be retained in the

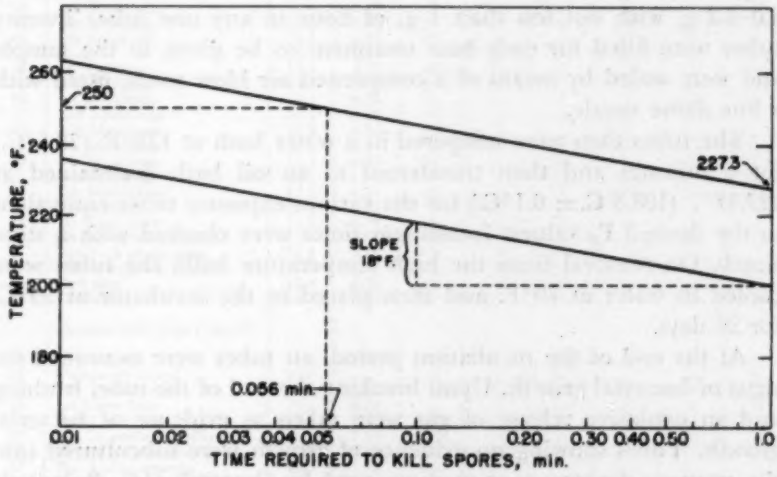

Fig. 1. Sections of thermal death time curves having z = 18° F. The destruction time is plotted on a logarithmic scale. The upper curve is for a thermal death-time of one min. at 227.3° F. One minute at a constant temperature of 227.3° F. is equivalent in lethal value to 0.056 min. at a constant temperature of 250° F. The lower curve is for a thermal death-time of one min. at 200° F.

bath at 227.3°F. to give the equivalent F_0 values were readily calculated. Reference to Fig. 1 shows that one minute at a constant temperature of 227.3°F. is equivalent to 0.056 minutes at a constant temperature of 250°F. It follows therefore that 0.5 minutes at 250°F. is equivalent to 0.5/0.056 or 8.93 minutes at 227.3°F. Other F_0 values were similarly calculated. Applying the correction factor of 1.98 to allow for heating and cooling, the following times were obtained:

F_0 value	*Equiv. at constant temp. of 227.3° F.*	*Correction factor*	*Total exposure time*
min.	*min.*	*min.*	*min.*
0.5	8.93	1.98	10.91
1.0	17.86	1.98	19.84
1.5	26.80	1.98	28.78
2.0	35.75	1.98	37.73

The thermal death-time tubes (Kimble No. 45050, 10 mm. x 75 mm., test tubes with lip) were filled with a paste made up as follows: Approximately 200 ml. of distilled water containing 7 g. of dehydrated liver infusion were mixed with 100 g. of sample in a vacuum mixer (11) to keep air bubbles to a minimum. Enough water was added to produce paste of the necessary fluidity. The paste was dispensed to the tubes by means of a special measuring pipette, the amount of paste dispensed, depending on the amount of water added, was usually 3.0–3.2 g. with not less than 1 g. of flour in any one tube. Twenty tubes were filled for each heat treatment to be given to the sample and were sealed by means of a compressed air blow torch, fitted with a fine flame nozzle.

The tubes then were tempered in a water bath at 175°F. (79.5°C.) for 5 minutes and then transferred to an oil bath maintained at 227.3F°. (108.5 C.± 0.1°C.) for the various exposure times equivalent to the desired F_0 values. Immersion times were checked with a stopwatch. On removal from the high temperature bath, the tubes were cooled in water at 70°F. and then placed in the incubator at 55°C. for 28 days.

At the end of the incubation period, all tubes were examined for signs of bacterial growth. Upon breaking the end of the tube, frothing and an explosive release of gas were taken as evidence of bacterial growth. Tubes showing no evidence of growth were subcultured into the tryptone-dextrose-agar medium used by Omernik (11). Relatively large inocula were used (about 0.3 g.) and the subculture tubes were incubated at 55°C. for 7 days. Bacterial growth was evidenced by heavy turbidity, acid production, or gas formation in the subculture

tubes. Growth either in the thermal death-time tubes or the subculture tubes was recorded as positive evidence of survival of spores in the flour suspension.

Results and Discussion

The thermophilic spore counts and heat resistance data obtained on the 38 samples are given in Table I.

THE THERMOPHILIC SPORE COUNTS AND THE HEAT RESISTANCE OF THERMOPHILIC SPORES PRESENT IN WHEAT AND MILL PRODUCTS

No.	Sample Description	Composition[1]		Thermophilic Spores per g.			Relative Heat Resistance No. of Tubes Showing Growth Out of 20 Treated and Incubated at F_0[2]		
		Protein %	Ash %	Aerobic Spores Total	Aerobic Spores Flat Sours	Anaerobic Spores[3]	0.5	1.0	1.5
	Durum 1948 crop ("old")								
1	Dry wheat	12.3	1.74	70	33	4	8	8	2
2	Tempered wheat	12.1	—	27	3	0	4	1	0
3	No. 1 Semolinas	11.2	0.56	22	3	1	5	2	0
4	1st Clear flour	12.8	1.20	30	15	0	4	3	1
5	2nd Clear flour	13.6	1.53	37	10	2	4	3	0
	Bread Wheat								
6	Dry wheat	12.0	1.66	8	5	0	0	2	0
7	Tempered wheat	12.4	1.65	3	3	0	0	0	0
8	1st Break stk.	12.2	1.72	15	10	0	2	0	0
9	2nd Break stk.	12.5	1.89	35	15	3	3	1	0
10	3rd Break stk.	13.4	2.49	67	23	2	6	3	0
11	4th Break stk.	14.8	3.92	27	7	2	3	0	0
12	5th Break stk.	14.5	5.71	18	8	2	0	0	0
13	Bran	13.1	6.47	5	2	2	0	0	0
14	1st Tailings	13.4	2.76	43	29	3	5	5	0
15	2nd Tailings	14.9	3.98	13	3	3	0	0	0
16	1st Middlings	10.1	0.38	11	0	0	0	0	0
17	2nd Middlings	10.4	0.45	8	8	2	0	0	0
18	3rd Middlings	10.8	0.54	10	8	2	3	0	0
19	4th Middlings	10.0	0.67	5	2	0	0	0	0
20	5th Middlings	11.1	0.87	15	8	2	2	0	0
21	6th Middlings	12.3	1.44	8	5	0	0	0	0
22	7th Middlings	12.5	1.51	12	7	0	2	0	0
23	Patent flour	10.8	0.36	8	7	0	0	0	0
24	1st Clear flour	13.8	0.79	5	2	0	0	0	0
25	1st Low grade flour	13.7	2.07	3	2	7	0	0	0
26	2nd Low grade flour	13.9	2.26	8	3	5	0	0	0
27	3rd Low grade flour	13.9	2.47	5	2	5	2	0	0
28	Red dog	15.6	2.56	63	39	3	6	4	0
29	1st Sizing	11.7	1.33	33	23	2	3	3	0
	Durum 1949 crop ("new")								
30	Dry wheat	11.2	1.53	8	6	0	0	0	0
31	Tempered wheat	11.7	—	2	0	0	0	0	0
32	No. 1 Semolinas	11.6	0.56	7	3	0	0	0	0
33	1st Clear flour	13.1	0.56	3	2	0	0	0	0
34	2nd Clear flour	14.2	1.42	17	13	0	2	0	0
35	No. 30 surface sterilized	11.2	1.53	0	0	0	0	0	0
36	No. 30 surface sterilized	11.2	1.53	2	0	0	0	0	0
37	No. 1 after storage	12.3	1.74	59	47	5	—	—	—
38	No. 37 surface sterilized	12.3	1.74	8	2	2	—	—	—

[1] Expressed on 14.0% moisture basis.
[2] Samples numbered 1 to 5 inclusive were processed at Fo = 2.0 and incubated; none of the tubes showed any growth.
[3] Probable least number.

The numbers of thermophilic spores in these wheats and wheat products were small; the maximum total count was 74 per gram and the thermophilic anaerobe count was always less than 10 per gram. Likewise, the heat treatments required for sterilization were low. Approximately 50% of all the samples examined were sterilized by heat treatments equivalent to $F_0 = 0.5$ or less, despite the fact that these samples included raw wheats and many low grade stocks. Only two samples contained viable cells after receiving a heat treatment equivalent to $F_0 = 1.5$ and none at $F_0 = 2.0$. The higher the initial spore count, the longer was the sterilization time required in the majority of cases. This observation would indicate that survival time was a function of initial population rather than due to the presence of some extremely resistant species.

The cleaning, washing and tempering operations markedly decreased the spore count of the wheats and the remaining microorganisms appear to be located rather close to the surface. The very low grade stocks containing bran, germ, and scutellum particles in large amounts (red dog, first sizing, first tailings and the like) were appreciably higher in spore counts than the whole wheat, higher grade flours and middlings, and the bran. The patent flour, which consists of the inner portions of the endosperm, and the first clear flour were both quite low in thermophilic spore count. The spore count of the break stocks increased up to the third break and then decreased as the removal of the endosperm became more complete.

The mill products most likely to be used in canned foods are the durum semolinas (Nos. 3 and 32), the first and second clear durum flours (Nos. 4, 5, 33, 34) and the lower grade bread wheat flours namely the fifth, sixth and seventh middlings flours (Nos. 20–22) first clear flour (No. 24), first, second and third low-grade flours (Nos. 25–27) and the first tailings flour (No. 14). With only a few exceptions these wheat products had a low total spore count and were sterilized by heat treatments equivalent to $F_0 = 0.5$ or less. Samples of durum semolina, first and second durum clears and first tailings bread wheat flour required a heat treatment equivalent to $F_0 = 1.0$. Of the entire group of samples, only one (durum first clear) showed any survival of spores after a heat treatment equivalent to $F_0 = 1.5$.

These sterilization requirements are much lower than those reported by Omernik (11) on 11 samples of soy flour; none of the samples she tested were sterilized by a treatment of $F_0 = 2.0$ and two samples contained viable spores after treatments of $F_0 = 4.0$. If the samples employed in the present study can be regarded as representative of the flours produced by a Northwestern United States mill, it

would appear that, in contrast to soy flours, they do not require sterilizing treatment before being used in canned products. This conclusion is justifiable when one considers the heat resistance of the spores involved rather than spore counts per unit volume.

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FLAVOR OF BREAD[1]

J. C. BAKER, H. K. PARKER, and K. L. FORTMANN [2]

ABSTRACT

The origin of bread flavor has been classified into three broad groups, namely, ingredients, fermentation, and baking. It has been demonstrated that fermentation and the formation of a brown crust are essential for satisfactory flavor.

Ethyl alcohol, acetaldehyde, fusel oil, pyruvic aldehyde, diacetyl, furfural, acetic acid, along with iso-aldehydes, ketones and esters have been identified in condensates of the vapors from bread baked after normal and excessive fermentations. The quantities of the individual constitutents in the condensate varied from a trace to 12%.

Bread flavor can be improved in practice by avoiding too fine a crumb and excessively large volume, by altering baking to affect production of aldehydes and thus control the extent of browning, by increasing flavor components arising from amino acids and by retention of volatile flavor components in the crumb through faster baking and quicker wrapping.

The flavor of bread, mild as it may be considered, has universal appeal to the human appetite. The taste of freshness in bread is especially desired and so widely sought that breads are being made increasingly soft to suggest this quality. If commercial bread could be made to retain its fresh flavor there might be an increase in bread consumption. Frozen doughs and the merchandising of partially baked bread are approaches to that end. Their success is due to taste appeal.

The flavor of bread can be considered as originating from three broad sources, i. e., ingredients, fermentation and baking.

The components of bread are basically flour, water, salt and yeast. Well milled flour from sound wheat contributes its mild flavor which should not be confused with that found in musty products, whole wheat or improperly cleaned wheat. Starch and enzymes of flour and the action of saliva are largely responsible for the sweetness in bread made from these basic components. Salt is a most important taste ingredient which helps develop the qualities of all flavors. Yeast by itself contributes little to flavor unless added in excess. Generally, a yeasty flavor, as distinguished from fermentation, is not desirable. To these basic ingredients of bread are added many other components, the most important of which is sugar. Milk, malt and germ may possibly change bread flavor slightly but they are generally used in small amounts and their effects on flavor are rather minor. When fats are added to bread, they may also yield various flavors.

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Fermentation and baking flavors, together are the chief source of bread's appeal. One can mix bread ingredients to form a dough, immediately proof and bake, such as has been done with the no-time-chlorite method, and obtain a good-appearing product with little taste appeal (3). Fermentation of the dough is required to obtain the desired flavor.

A well-fermented dough may be baked electrically by internal resistance without formation of a crust but this yields a mild, unappealing bread crumb (2). These experiments prove that to have full flavor, the dough must not only be fermented but also form a crust during baking.

Fermentation may be carried out separately from flour by permitting yeast to act on a sugar solution containing yeast nutrients, and after suitable action has occurred, the fermented liquid may be used to form a dough which can be immediately proofed and baked. Such bread has full flavor appeal. This experiment indicates that the flavor effects of fermentation are from the action of yeast on sugar rather than on other flour components.

A further informative experiment on the origin of bread flavor came from baking an over-fermented sponge to which salt was added just before proofing. Such dough, baked at regular oven temperature and time, yielded bread with a colorless crust in which no appreciable browning occurred. Its flavor was not distinguishable from the same dough baked without the formation of crust. Hence it appears that the browning reaction is also required to give full flavor. When sugar was added to the above dough before baking, the crust browned and the full flavor of bread was obtained.

These experiments show that sugar is the source of flavor obtained both from fermentation and from the browning reaction. Sugar can be contributed by the flour through the sugars it contains or through their production by amylase action; sugar can also be added in the baking formula to intensify the effects.

These results lead to a much more difficult question. What are the reactions that produce these flavor effects and what compounds are responsible? The object of this paper is to present some new approaches to a subject which is of great importance to the baking industry, namely, the flavor of bread.

Materials and Methods

Two experimental techniques were used in gathering data for the present study. The first involved collecting the condensate from

oven vapors and subsequently analyzing it for the presence of flavor components. The second technique involved measuring the amount of volatile reducing substances in bread crumb after the method of Farber (6).

Condensate from Oven Vapors. An electrically heated laboratory oven was loaded with loaves to capacity (12 lbs.). The oven vent was connected by a glass tube to an aspirator. As the baking proceeded, some of the gases formed were led through a series of dry ice traps to condense the vapors. The solution resulting from melting and combining the condensates was then analyzed.

Most compounds were identified qualitatively and quantitatively by colorimetric methods as outlined by Snell and Snell (10). These included ethyl alcohol (by specific gravity and Schiff's reagent); amyl alcohol (by salicylic aldehyde); acetaldehyde (by Schiff's reagent); fusel oil (by aldehydes); total acidity (by titration); furfural (by aniline acetate); pyruvic aldehyde (by pyrrol and hydrochloric acid); acetone and acetoacetic acid (by mercuric cyanide reagent); methyl ethyl ketone (by alkaline salicylic aldehyde). Diacetyl was determined by method of Visser't Hooft and de Leeuw (12).

Volatile Reducing Substances in Bread Crumb. Farber (6) in 1949 published a method for determining volatile organic substances. Among other tests, he passed air through bread crumb and then through a standard permanganate solution. The amount of permanganate reduced gave a measure of the volatiles in the crumb.

The apparatus and procedure used in this test were essentially the same as those employed by Farber. A measured volume of purified air was blown through a given weight of bread crumb supported on a fritted glass disk in an all-glass chamber. The exit gases were passed through two test tubes in series, each containing 10 ml. of O.1*N* potassium permanganate in 1.0 *N* sodium hydroxide. The amount of reduction of the permanganate after absorption of the volatiles aerated from 2 g. of bread crumb at an air rate of one liter per minute for 10 minutes, was determined iodimetrically and expressed as micro-oxidation equivalents of volatile reducing substances (V.R.S.).

Results

Oven vapors from normal and from overfermented sponges were collected and analyzed to find out why the latter bread lacks flavor, in comparison to that from normal sugar-containing sponges.

Table I shows the components, other than water, of these condensates in the order of the amounts present. Ethyl alcohol was the

TABLE I

ANALYSIS OF OVEN CONDENSATE OBTAINED DURING BREAD BAKING

Compounds Found	Sugar Present	Sugar Gone
	%	%
Alcohol	12.00	11.00
Acetaldehyde	0.06	0.02
Iso-alcohols (fusel oil)	0.04	0.04
Acids as acetic	0.02	0.02
Furfural	0.01	none
Pyruvic aldehyde	0.01	none
Ketones	+	+
Diacetyl	+	none
Esters	trace	trace
Iso-aldehydes	trace	none

chief substance, followed by acetaldehyde, fusel oil, and acid in both doughs. Furfural[3], pyruvic aldehyde, diacetyl and isoaldehydes were found only in the condensates from the doughs containing sugar. These substances all have strong and suggestive odors. Furfural in these dilutions is similar to the odor of roasting grain. Pyruvic aldehyde has the characteristic odor of caramel or burning toast. Diacetyl, which is found in butter, has a distinctly pleasing aroma at great dilutions, and is suggestive of the odor from baking bread.

Iso-aldehydes (8) are present in very small amounts, but doubtless lend their potent quality to the baking odor which results from the combination of all these substances.

Most of the aldehydes listed are very reactive and unstable. Doubtless, portions were lost before they could be collected and analyzed; pyruvic aldehyde in particular disappeared quickly. Synthetic solutions of the substances shown in Table I, when sprayed in the air, are very similar to the baking odor, but do not carry its full strength. Fortification with pyruvic aldehyde and diacetyl improved the result and produced a fairly good imitation.

When bread crumb was aerated in a closed container and the vapors collected, greatly reduced amounts of ethyl alcohol were obtained, as compared to that obtained from oven vapors. Traces of amyl-alcohol, diacetyl, furfural and pyruvic aldehyde also were identified. No trace of acetaldehyde or acetone was found. These results show that the highly volatile substances in the oven vapor condensate are baked out quickly from bread.

When bread of good quality is baked, the dough is in an active fermenting condition. The reactions of fermentation are in full play

[3] Colorimetric tests indicated the presence of furfural but little or no hydroxy-furfural.

when suddenly arrested by the oven heat. There should be present in the baked bread all of the less volatile intermediate compounds involved when sugar is fermented by yeast.

TABLE II

FLAVORY SUBSTANCES IN BREAD DUE TO FERMENTATION AND FORMED DURING BAKING

Volatile	Less Volatile	Low Volatile
Alcohol	Iso-alcohols	[1]Melanoidins (crust brown)
[1]Pyruvic aldehyde	Acetic Acid	Dihydroxyacetone
Diacetyl	Pyruvic Acid	Ethyl Succinate
[1]Iso-aldehydes	[1]Furfural	Lactic Acid
	Acetoin	Succinic Acid
	Butylene Glycol	
	Ethyl Lactate	

[1] Formed during baking.

Table II gives a list of the substances which are believed to be present in bread as a result of fermentation and baking and which may characterize or add flavor. Those formed during baking are indicated by a footnote.

Succinic acid is formed during fermentation from glutamic acid (7). Lactic acid can be formed by bacteria or by the addition of water to pyruvic aldehyde. Acetic acid may form from the action of bacteria on alcohol in the presence of oxygen. These acids are ordinarily present in bread in such small amounts that they have but little effect on flavor; however, in dough they may form ethyl esters.

Ethyl acetate has a low boiling point (77°C.). It is probably completely distilled from bread during baking and does not contribute to flavor. Ethyl lactate, boiling point (154°C.) has the odor of rum. Ethyl succinate, boiling point (218°C.) has a faint, pleasant odor. These esters may contribute bouquet to bread flavor.

The compounds remaining in the crumb after baking are for the most part, of low volatility and contribute more to taste than to odor. To identify their individual effects is a difficult task, but one can describe their combined action as the fermentation-baking flavor effect.

If bread crumb is aerated with humid air until all volatiles are removed, the flavor remaining in the still soft crumb will carry the effect of the non-volatiles produced by baking and fermentation. It has a taste suggestive of stale bread which indicates that this quality may be due to the loss of volatiles; however, during the long aeration (four hours) required to remove the volatiles, oxidation may have occurred. When such aerated crumb is compared with cooked, unfermented dough, the presence of these non-volatile compounds can be

noted as providing a slightly pleasant sweetness. Even here it is impossible to say that this quality is entirely due to non-volatiles, for acetoin and butylene glycol are only slightly volatile; acetoin is always subject to oxidation with the formation of traces of diacetyl, which has a potent butter-like flavor as shown by Visser't Hooft and de Leeuw (12). Besides sugars, other soluble, organic substances contribute to the development of bread flavor. Free amino acids have long been known as a source of fusel oil in fermented or distilled liquors (7). Likewise they play an important part in the quality of bread flavor. If leucine is added to a fermenting sponge in amounts exceeding 50 p.p.m., the bread has an aromatic, cheese-like unappetizing flavor. Leucine and iso-leucine are the source of the iso-amyl alcohols formed during fermentation. If valine is added, iso-butyl alcohol, which has a mild and less disagreeable flavor than iso-amyl alcohols, is produced. Phenylalanine added to dough produces an odor in bread which is reminiscent of roses or pansies. Small amounts of various amino acids may give pleasing flavor effects. Sullivan (11) has shown that traces of all ordinary amino acids are present in flour, hence one can suspect variations in bread flavor due to the amount and nature of these soluble constituents of flour. Possibly, flours milled from different wheat varieties or of wheats produced under various environmental conditions might give breads which differ in flavor.

Another interesting effect of amino acids is in the browning reaction which is so important to flavor (8). Sugars will not by themselves brown appreciably at the temperature of approximately 150°C. which is reached in the crust of bread during baking (4). The escaping interior gases keep the crust cool and prevent it from reaching higher temperatures. If amino acids or ammonia are added to sugar, then browning will take place at crust temperatures with the occurrence of many side reactions and at a lower temperature than with sugar alone (8). Amino acids can provide the nitrogen required for the formation of colored condensation products with pyruvic aldehyde (methyl-glyoxal); in doing so they are de-aminized to form aldehydes. Leucine and iso-leucine thus react to yield iso-amyl-aldehydes (8).

To summarize, some of the amino acids are reduced by fermentation to an alcohol or by browning to an aldehyde, both of which carry potent odors and flavors. The amino groups involved in the browning reaction may be of wider origin than from free amino acids. Casein reacting with sugar loses its free amino groups (9).

The effect of mixing on browning and flavor is an interesting one. If doughs made from identical formulas are slightly mixed, mixed to the optimum, or overmixed, the crust of the baked bread will progress

from a light, underbrowned, grayish crust to a full, deep brown with the overmixed dough. A corresponding change in flavor is noted. The undermixed sample is suggestive of the flavor of flour or raw dough, while the overmixed one has a full, rich appealing flavor. The reasons for this effect are not fully apparent and may involve crust thickness; the effect may be associated with the unfolding of the protein micelle during mixing to expose reactive amino groups which take part in the browning reaction with release of flavor.

Browning reactions contribute largely to give the characteristic flavor of such products as hard rolls, browned cereals, toast and crackers. In these products most of the volatiles produced by fermentation have been lost during baking or toasting. The permanent, flavory component is likely the bitter unknown polymer of pyruvic aldehyde with nitrogen compounds. The odor noticed while toasting is largely due to furfural and the more volatile and quickly lost pyruvic aldehyde.

Volatile aldehydes are also responsible for the crusty flavor carried into bread crumb. Hot bread, as it comes from the oven, has a maximum content of volatile substances in the dehydrated crust, which are rapidly drawn to the interior as the steam and alcohol of the crumb condense and create a negative pressure in the interior of the loaf.

The staling of bread flavor, which is an independent and different reaction from crumb firming, is an interesting study. Condensates of oven vapor, upon standing, develop a stale odor. Crustless baked breads do not develop stale flavor appreciably (2); hence it appears that the stale flavor may be associated with the volatiles that are drawn from the crust into the crumb upon cooling. These compounds are probably aldehydes of various reactivities. Pyruvic aldehyde is very reactive and quickly disappears. Furfural is the most stable of these aldehydes, although it may oxidize to furoic acid which has no odor. The iso-aldehydes are fairly reactive and may slowly oxidize to the corresponding acids. Iso-valeric acid has a very potent, disagreeable, rancid, cheese-like odor. Iso-butyric acid has an unpleasant odor, which is more disagreeable than that of butyric acid. The properties of these aldehydes and their presence in crust lead to the suggestion that the development of stale flavor may be due to their slow oxidation to unpleasant volatile organic acids.

Butterworth (5), in 1935, made a comprehensive study of the relation of bread making to bread flavor. He concluded that the amount of oxidation and the extent of proofing were the chief variables in the baking process which influenced flavor. Absence of oxidation with

oxidizing improvers or improper proof, especially overproof, gave the poorest results.

These two conditions produce bread that is porous; that is, air can be easily drawn through the crumb. Some years ago Baker (1) built a machine for studying this effect and found that breads which resisted the flow of air retained their flavor. Air passes more easily through bread crumb when the cells are greatly torn during expansion in the oven, that is, by the oven spring. This tearing is most affected by the amount of proof and by the ability of the dough to yield to the expansion without breaking, as in a properly developed dough. Apparently the flavor is thus influenced by the retention of volatiles.

The effect of baking conditions on the amount of volatile reducing substances (V.R.S.) remaining in bread is shown by the data in the following table:

Type of Bake	*Micro-oxidation equiv. V.R.S.*
Normal bake	420
Overbake	365
Underbake	460
Quick bake	440
Slow bake	390

Variations in the amount or speed of baking causes a considerable difference in the volatiles retained in bread. The principal substance responsible for variation is ethyl alcohol, but one must realize that to accomplish its removal large amounts of water must be evaporated as steam, in this way much of the less volatile flavory substances would be carried off. Probably, as more alcohol remains in the bread crumb, more of the other volatiles also remain.

The effect of various treatments after baking on the volatile reducing substances is shown by the following data:

Treatment	*Micro-oxidation equiv. V.R.S.*
Out of oven, 15 min.	440
Out of oven, 1 hr.	420
Vacuum cooled, 7 min.	310
Exposed to air, 1 day	13
Wrapped, 1 day	411
Wrapped, 3 days	315
Wrapped, 9 days	103
Wrapped & frozen, 7 days	414

The quicker the bread is wrapped the greater is the retention of volatiles. In cooling, evaporation should be avoided as much as possible, as shown by the action of vacuum cooling. Exposure of sliced bread for one day, without wrapping, resulted in nearly complete loss of V.R.S. After wrapping, bread lost V.R.S. slowly but progres-

sively with time. There are now wrapping materials available such as polyethylene which will retain V.R.S. for the life of the bread. Freezing is a most successful method of retaining V.R.S.

Discussion

The question arises as to what can be done to improve bread flavor in practice. Bread production can be so handled as to change and retain the volatiles in the crumb by avoiding too fine a crumb and too much volume. Baking can be altered to affect the production of aldehydes and control the browning reaction. Browning reactions and crust flavor can be improved by the use of sugars in the baking formula.

Ammonium compounds may promote a brown color at lower crust temperatures and at the same time substitute for amino acids in the production of aldehydes. Flavor components arising from amino acids possibly may be increased by the addition of proteolytic enzymes. Enzymes must be used with caution, since they may possess unwanted proteolytic or lipolytic effects that will adversely alter taste toward cheesy or greasy qualities.

Volatiles and flavor arising from fermentation reactions can be increased by longer sponge time, more yeast and ample sugar. Stronger flours in the dough-up may offset the deleterious effect of more extensive fermentation in the sponge upon loaf volume and crumb properties. Volatiles can be retained in the crumb by faster baking and by proper, quicker wrapping.

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THE EFFECT OF FERTILIZER APPLICATION AND ANALYSIS ON CERTAIN CRITERIA OF MALTING QUALITY IN BARLEY[1,2]

K. J. Frey and L. S. Robertson[3]

ABSTRACT

The application of commercial fertilizers of varying composition to five varieties of malting barley resulted in an average increase in the percentage of malt extract of the malted grain of 0.9% in 1949 and 0.4% in 1950. The protein content of the barley grain was not appreciably altered by the application of fertilizer. In 1949 diastatic power was decreased by each fertilizer used. This was not the case in 1950. The effect of fertilizer composition on kernel size was not consistent from year to year.

When fertilizer is applied to a barley crop, one purpose is to increase the yield of grain per acre. Any effects which the fertilizer may have upon malting quality, either beneficial or detrimental, have not usually been determined. According to some reported experimental results (3, 4, 5, 6) fertilizers may have a slight effect on malting quality. It has been demonstrated in Wisconsin, at least in certain years, that the quantity of extract from barley malt was increased from 1 to 2% when fertilizer was applied to the barley crop. Also, protein content and diastatic power were affected by fertilizer application (5, 6). In all of the Wisconsin experiments the application of fertilizers, especially those containing nitrogen, tended to increase kernel size. Ayr and Mayr (2) found that the application of calcium and phosphorus improved the malting quality of the barley grain, while potassium depressed protein content, and nitrogen affected malting quality adversely.

Meredith, *et al.* (8) reported that the application of fertilizer to barley affected malting quality more when the barley was planted early than when planted late.

A summary of the work reported to date shows, first, that fertilizer applied to barley has only slight effects on the malting quality of the barley, and second, the results have been variable from location to location.

The purpose of the present study was to determine the effect of

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various fertilizers on the malting quality of barley varieties. The criteria used to measure malting quality were: (1)[4] malt extract percentage, (2) protein content of barley, (3) diastatic power of malt, and (4) one-thousand kernel weight of the grain.

Materials

The lots of barley were grown on soil classed as a Brookston silt loam in Saginaw and Tuscola counties of Michigan in 1949 and 1950, respectively. In 1949 four barley varieties, Wisconsin 38, Montcalm, Moore and O.A.C. 21, were grown and in 1950, Bay was included. The five fertilizer treatments used were: Check, or no treatment, and 0–16–0,[5] 0–16–16, 4–16–8, 4–16–16 applied at the rate of 500 pounds per acre.

Samples for malting determinations were obtained by compositing the threshed grain of each variety-fertilizer treatment from four replications. The samples were malted by the George J. Meyer Malt and Grain Corporation of Buffalo, New York in 1949, and by the Joseph Schlitz Brewing Company of Milwaukee, Wisconsin in 1950.[6] The malts obtained from the barley samples were analyzed for extract percentage and diastatic power according to the methods of the American Society of Brewing Chemists (9). Protein determinations, employing a modification of the Kjeldahl procedure (9), were made on the barley grain.

Results

The extract percentages of malt from the barley samples given in Table I indicate that variety was a more important factor in determining extract percentage than was fertilizer treatment. Montcalm variety was highest and Wisconsin 38 was lowest in extract percentage in both years. The differences in extract percentage between fertilizer treatments were significant in 1949. When the sum of squares due to the "between fertilizers" source was broken down, the differences between no fertilizer and fertilizer were significant, but the variation among the various fertilizers was not. The average increase in extract percentage due to fertilizer application was 0.9%.

The mean square due to "between fertilizers" was not significant

[4] The data for extract and protein percentages and diastatic power are expressed on a dry basis.

[5] The fertilizer analysis 0-16-0 refers to the percentage of N, P_2O_5, K_2O, respectively, in a lot of fertilizer.

[6] These companies are members of the Midwest Barley Improvement Association, which Association arranged for these malting tests as a part of the cooperative project in barley improvement at the Michigan Agricultural Experiment Station.

in 1950. By using the questionable practice of breaking down a non-significant sum of squares, it was found that fertilizer application significantly increased the extract percentage also in 1950. The average increase was 0.4%.

TABLE I

PERCENTAGES OF MALT EXTRACT OBTAINED FROM BARLEY VARIETIES WHICH WERE GROWN ON PLOTS RECEIVING DIFFERENT FERTILIZER TREATMENTS

Variety	Fertilizer Treatments					Mean
	None	0-16-0	0-16-16	4-16-8	4-16-16	
	%	%	%	%	%	%
1949						
Wisconsin 38	71.6	72.8	72.4	72.8	72.4	72.4
Montcalm	76.9	77.4	77.4	77.4	77.0	77.2
Moore	74.1	75.7	75.6	75.4	75.4	75.2
O.A.C. 21	76.0	76.6	76.4	76.8	76.4	76.4
Mean	74.6	75.6	75.5	75.6	75.3	
1950						
Bay	76.1	76.8	76.8	76.9	76.9	76.7
Wisconsin 38	73.4	74.1	74.0	74.9	74.8	74.2
Montcalm	77.6	78.2	77.6	78.3	77.8	77.9
Moore	76.5	76.8	76.5	76.8	76.4	76.6
O.A.C. 21	76.7	76.5	76.3	76.3	76.4	76.5
Mean	76.1	76.5	76.2	76.6	76.5	

ANALYSIS OF VARIANCE

Source	1949		1950	
	DF	Mean Square	DF	Mean Square
Between varieties	3	22.33**	4	88.10*
Between fertilizer treatments	4	0.64**	4	0.27
Check vs. fertilizers	1	2.28**	1	0.65*
Among fertilizers	3	0.09	3	0.14
Varieties x fertilizers (error)	12	0.06	16	0.10

*Denotes significance at 5% level.
**Denotes significance at 1% level.

The protein contents of the barley samples are given in Table II. The average protein percentage of all of the samples was 13.6 in 1949, while in 1950 the same varieties and treatments averaged only 9.7%

TABLE II

PROTEIN PERCENTAGES OF BARLEY VARIETIES WHICH WERE GROWN ON PLOTS RECEIVING DIFFERENT FERTILIZER TREATMENTS

Variety	Fertilizer Treatment					Mean
	None	0-16-0	0-16-16	4-16-8	4-16-16	
	%	%	%	%	%	%
1949						
Wisconsin 38	14.8	13.5	13.7	14.1	14.5	14.1
Montcalm	13.2	12.7	12.9	13.3	13.6	13.1
Moore	14.8	13.5	14.1	13.7	14.4	14.1
O.A.C. 21	13.8	13.8	12.8	13.0	13.5	13.4
Mean	14.1	13.3	13.3	13.5	14.0	13.6
1950						
Bay	10.1	9.6	9.8	10.1	9.8	9.9
Wisconsin 38	9.6	9.4	9.5	9.4	9.4	9.5
Montcalm	9.2	9.1	9.5	9.4	9.8	9.4
Moore	9.7	9.5	9.4	9.4	9.6	9.5
O.A.C. 21	9.9	10.5	10.4	10.8	10.4	10.4
Mean	9.7	9.6	9.7	9.8	9.8	9.7

ANALYSIS OF VARIANCE

Source	1949		1950	
	DF	Mean Square	DF	Mean Square
Between varieties	3	1.15*	4	0.87**
Between fertilizer treatments	4	0.46	4	0.03
Varieties x fertilizers (error)	12	0.17	16	0.06

*Denotes significance at the 5% level.
**Denotes significance at the 1% level.

protein. The difference in protein percentage between the two years is not a manifestation of the usually accepted negative relationship between yield per acre and protein percentage in the cereal grain crops since the average yield of the barley in 1949 was 45.0 bushels per acre, or approximately 20% higher than the 36.2 bushel yield in 1950. In both years there was an abundance of well distributed rainfall during June and July, so the difference cannot be explained on this basis either. The most logical explanation for this difference of 3.9% in protein content of the barley between the two years seems to be in the crops grown on the field previous to the planting of the

TABLE III

DIASTATIC POWER IN DEGREES LINTNER OF THE MALT OBTAINED FROM BARLEY VARIETIES WHICH WERE GROWN ON PLOTS RECEIVING DIFFERENT FERTILIZERS TREATMENTS

Variety	Fertilizer Treatment					Mean
	None	0-16-0	0-16-16	4-16-8	4-16-16	
	L°	L°	L°	L°	L°	L°
1949						
Wisconsin 38	105	95	91	95	100	97
Montcalm	190	191	183	193	197	191
Moore	146	121	127	134	128	131
O.A.C. 21	145	131	129	126	127	132
Mean	146	134	132	137	138	
1950						
Bay	101	93	93	104	100	98
Wisconsin 38	76	73	76	78	72	75
Montcalm	97	101	105	109	106	104
Moore	83	81	76	76	79	79
O.A.C. 21	88	91	93	100	88	92
Mean	89	88	89	93	89	

ANALYSIS OF VARIANCE

Source	1949		1950	
	DF	Mean Square	DF	Mean Square
Between varieties	3	7565.0**	4	751.5**
Between fertilizer treatments	4	115.0	4	24.3
Check vs. fertilizer	1	387.0**	—	—
Among fertilizers	3	24.3	—	—
V x F (error)	12	30.0	16	15.6

*Denotes significance at the 1% level.
**Denotes significance at the 5% level.

barley. In 1949 the barley was planted on a plowed alfalfa sod while in 1950 it was planted on sugar beet land. The alfalfa land had a better soil structure and probably a higher nitrogen content than the sugar beet land, thus resulting in more nitrogen being available and causing a higher protein percentage in the succeeding barley crop.

The only significant source of variation in protein percentage in both 1949 and 1950 was between varieties.

TABLE IV

WEIGHT PER 1000 KERNELS OF BARLEY VARIETIES GROWN ON PLOTS RECEIVING DIFFERENT FERTILIZER TREATMENTS

Variety	Fertilizer Treatment					Mean
	None	0-16-0	0-16-16	4-16-8	4-16-16	
	g.	g.	g.	g.	g.	g.
1949						
Wisconsin 38	34.7	33.6	34.0	33.8	34.3	34.1
Montcalm	35.0	34.9	34.7	34.1	35.0	34.7
Moore	36.1	35.1	35.3	35.5	36.2	35.6
O.A.C. 21	33.0	32.8	32.7	32.8	33.3	32.9
Mean	34.7	34.1	34.2	34.0	34.7	
1950						
Bay	33.3	34.4	33.5	35.4	35.4	34.4
Wisconsin 38	31.7	32.5	33.6	34.5	35.7	33.6
Montcalm	33.3	34.8	35.0	36.4	36.2	35.1
Moore	33.5	34.0	35.3	35.1	35.4	34.7
O.A.C. 21	34.0	34.5	35.5	36.4	36.2	35.5
Mean	33.2	34.0	34.6	35.6	35.8	

ANALYSIS OF VARIANCE

Source	1949		1950	
	DF	Mean Square	DF	Mean Square
Between varieties	3	6.56**	4	2.30**
Between fertilizer treatments	4	0.43**	4	5.93**
Check vs. fertilizers	—	—	1	13.46**
Nitrogen vs. no nitrogen	—	—	1	9.38**
Within fertilizers	—	—	2	0.45
Variety x fertilizer (error)	12	0.06	16	0.26

**Denotes significance at 1% level.

The diastatic powers of the barley samples are given in Table III. Variation between varieties was significant in each year with Montcalm and Wisconsin 38 varieties being consistently highest and lowest, respectively, in diastatic power. In 1949 the application of fertilizer caused a significant mean reduction of 11.0° in diastatic power in the barley. However, it seemed to matter little what fertilizer was applied for each caused about the same reduction.

The variety Montcalm did not show the marked reduction in diastatic power that the other varieties did when fertilizer was applied.

The weights per thousand kernels for the barley samples are given in Table IV. The variation between varieties was significant in both years. In 1949 the weight per thousand kernels was highest for Moore and lowest for O.A.C. 21, while in 1950 O.A.C. 21 was highest and Wisconsin 38 was lowest. This shift in varietal rank during the two years was similar to that of protein percentage.

The application of fertilizer in 1950 resulted in a significant increase in kernel weight of 1.8 g. per thousand kernels. Fertilizers carrying nitrogen caused a 1.4 g. per thousand kernels greater increase than did those containing no nitrogen. In the 1949 test, variation between fertilizer treatments was significant; the check and 4–16–16 fertilizer treatments gave the largest kernel size.

Discussion

According to Meredith, *et al.* (8) one of the most important criteria of good malting quality is a high extract percentage. From this standpoint the application of fertilizer to the barley plots in this study was beneficial by increasing the extract percentage.

The protein content of the barley samples was affected very little by fertilizer application. This is contrary to the popular belief in many barley growing areas that the application of fertilizers containing nitrogen will result in barley undesirably high in nitrogen content. However, the possible explanation of this is that these experiments were conducted in a humid area. Excess nitrogen applied without phosphate and potash, or in improper balance with these elements, or in combination with inadequate moitsure, might produce barley and malt higher in protein content than brewers desire.

The diastatic power was reduced in three of the four varieties of barley when fertilizer was applied in the 1949 test. From a practical standpoint, it is questionable whether this reduction was serious because the diastatic power of the fertilized samples was still sufficiently high for brewers' malt in every variety except Wisconsin 38. This reduction might be important for distiller's malt.

Malting barley grown in the midwest generally ranges between 31.0 and 34.0 g. per thousand kernels. In 1949 the application of 0–16–0, 0–16–16 or 4–16–8 fertilizer reduced kernel size somewhat, while in 1950 the application of fertilizer increased kernel size significantly.

Changes in malting quality due to fertilizer application, though significant in several cases, are nevertheless small in magnitude. In contrast, the yields were increased approximately 25% in 1950 when fertilizer was applied to the barley plots. This is in general agreement with the usual finding that the composition of plants is less subject to fluctuation caused by environmental factors than is total yield per acre. This does not imply that major compositional changes cannot be caused by large amounts of nitrogen or other elements, because it has been proposed that high available nitrogen was responsible for the high protein content of the barley from the 1949 experiment. However, the amount of nitrogen in any of the fertilizer applications was small in comparison to the amount available in the soil. For a more complete discussion of the effect of fertilizers on barley yields the reader is referred to Frey, *et al.* (7).

Another factor which must not be overlooked is the associated variation, sometimes positive and sometimes negative, between the various factors of malting quality. Anderson, *et al.* (1) have reported a positive correlation between total protein content and diastatic power in barley. Conversely they found a negative correlation between total protein content and kernel size and malt extract percentage. These relations are shown in the average values for the various fertilizer treatments in 1949 in Tables I, II, III, and IV. The check and 4–16–16 fertilizer treatments were lowest in malt extract percentage, and they were also highest in protein percentage, diastatic power, and kernel size.

The data from previous studies and those reported herein seem to justify the conclusion that the application of fertilizer to barley in humid regions if used within practical limits, causes relatively small changes in the factors related to malting quality. Probably the general fertilizer practices used for barley, at least in Michigan, do not materially harm the malting quality of the barley and in some respects they are beneficial.

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DETERMINATION OF CALCIUM IN FEEDS BY FLAME PHOTOMETRY[1,2]

Maxwell L. Cooley

ABSTRACT

The Beckman Flame Photometer has been successfully applied to the determination of calcium in feeds. The feed ash is dissolved in dilute acid, a great excess of phosphate added, the solution made up to an appropriate volume, atomized through the burner, and the intensity of the flame measured at 622 mμ.

Phosphate depressed the emission spectrum of calcium, particularly at 622 mμ. However, by addition of phosphate to bring the phosphorus to calcium ratio to 10:1 or more, no increase in depressing effect was noted.

Under these conditions, sodium, potassium, magnesium and manganese ions may be present in concentrations several times greater than that of calcium without affecting the accuracy of the determination. The interference of cobalt, copper and iron was small when present in concentrations lower than that of calcium as in the case in the ash of feeds.

The effect of sodium (strong emission band at 589 mμ) is mitigated by insertion of a didymium filter in the path of the light reaching the phototube.

The results obtained by this method compare well with those by the A.O.A.C. oxalate precipitation method.

Calcium is a necessary element in all livestock and poultry feeds. The interrelationship between calcium, phosphorus, and vitamin D (2) has long been recognized, and feed manufacturers must control the quantities of these nutritional factors in their products.

The use of flame photometry for the selective and expeditious determination of alkali metals is finding increasing acceptance (3,

[1] Manuscript received March 20, 1952. Presented at the Annual Meeting, April, 1952. Contribution from General Mills, Inc., Minneapolis, Minnesota.
[2] The data for this paper were obtained by use of the new model 9200 flame attachment for the Beckman DU spectrophotometer.

4, 5, 6), and the studies reported in this paper were undertaken to determine whether this technique could be employed for determining the calcium content of feeds and feedstuffs.

Factors Influencing Calcium Flame Emission

In order to obtain greatest value from flame measurements of calcium in feeds, a simple and rapid procedure was sought. This would provide for merely dissolving the ash of a feed sample in acid, making up to a certain volume, atomizing the solution through the burner, and reading the intensity of the flame at the desired wavelength. If such a method produced results which were within ±5% of the results obtained by the chemical determination of calcium (1), it would be considered sufficiently accurate for practical nutritional evaluations.

The optimum conditions for measurement of calicum flame radiation were investigated as follows: (1) most suitable wavelength for photometry, (2) most suitable oxygen and acetylene pressures, (3) range of concentration of calcium solutions, (4) interference of other ions, and (5) selection of best acid for solution of the feed ash. These factors are discussed in the order listed.

Development of Method for Determining Calcium in Feeds by Means of Flame Photometry

The emission maxima of calcium at 423, 554 and 622 $m\mu$ were examined. The maximum at 622 $m\mu$ was selected as most suitable because it is a strong calcium band. The interference of the emission bands of other elements present in the feed ash such as sodium, potassium, magnesium, manganese, cobalt, copper and iron is less at this wavelength. This maximum for calcium is in agreement with the work of Mosher, *et al.* (3).

A suitable flame for calcium measurements was obtained by using an oxygen pressure of 15 psi. and an acetylene pressure of 5 psi. These pressures give a blue flame free of yellow color, and provide for desirable atomization of the sample.

Investigation of solutions containing from 0 to 1000 ppm. of calcium led to the use of solutions varying between 0 to 100 ppm. of calcium as standards. The resulting curve, Fig. 6, is nearly linear and the range covers the calcium content of manufactured feeds.

Since the ash of feeding stuffs generally contains varying quantities of elements which interfere with the calcium emission spectra,

the effects of adding increasing amounts of a number of these ions to standard calcium solutions were investigated. The elements included those which are usually contained in feed ash in significant amounts and have relatively strong emission spectra such as sodium, potassium, magnesium and manganese as well as phosphorus which has no flame emission. Lesser elements such as cobalt, copper and iron also were examined.

Figure 1 shows the effect of adding varying quantities of sodium (as sodium chloride) to a series of 100 ml. volumetric flasks, each of which contained 10 mg. of calcium (as calcium chloride). The spectrophotometer was adjusted so that the solution containing only calcium (100 ppm.) when atomized under the conditions given in Fig. 1 produced 100% emission. The solutions containing the increments of sodium were then atomized and readings taken under the same conditions.

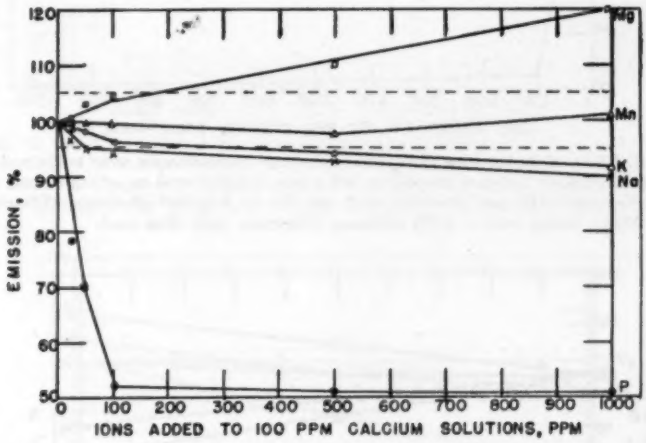

Fig. 1. Effect of Added Ions to Calcium Solutions—Measurements were made without the use of a filter. Conditions: Solution containing 100 p.p.m. calcium used to set instrument at 100% emission; Oxygen = 15 psi; Acetylene = 5 psi; Slit = 0.3; Red phototube; Wavelength = 622 $m\mu$; Flame background = 5.0% emission; no light filter used.

Likewise, a potassium (as potassium chloride) series of flasks, as well as a magnesium (as magnesium chloride), a manganese (as manganese chloride), and a phosphorus (as diamonium hydrogen phosphate) series were subjected to the same treatment as those containing sodium.

All readings in Fig. 1 were taken without the use of a didymium filter, while in Fig. 2 the operation was repeated using a didymium

filter. The maximum light absorption of this filter is 587 $m\mu$ while the emission maximum of sodium is 589 $m\mu$ – it still transmits freely at wavelengths for calcium and other metals.

Both Figs. 1 and 2 show phosphorus to be the most troublesome of the elements added, but the depressing effect on calcium emission

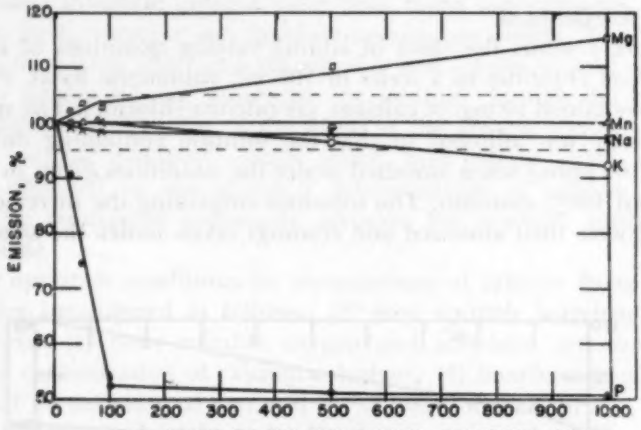

Fig. 2. Effect of Added Ions to Calcium Solutions–Measurements were made with the use of a filter. Conditions: Solution containing 100 p.p.m. calcium used to set instrument at 100% emission; Oxygen = 15 psi; Acetylene = 5 psi; Slit = 0.3; Red phototube; Wavelength = 622 $m\mu$; Flame background = 4.0% emission; Didymium light filter used.

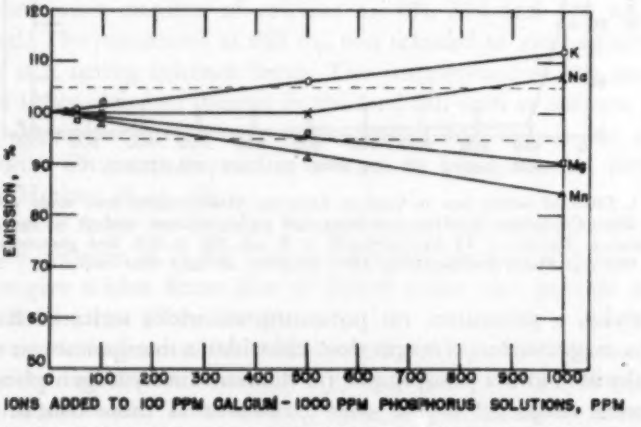

Fig. 3. Effect of Added Ions to Combined Calcium and Phosphorus Solutions–Measurements were made with the use of a filter. Conditions: Solution containing 100 p.p.m. calcium plus 1000 p.p.m. phosphorus used to set instrument at 100% emission; Oxygen = 15 psi; Acetylene = 5 psi; Slit – 0.5; Red phototube; Wavelength = 622 $m\mu$; Flame background = 18.0% emission; Didymium light filter used.

apparently levels off when the phosphorus is present in amounts 9 to 10 times greater than that of calcium. All per cent emission readings from 95 to 105% were considered within the limits of error desired. The "boxed in" area, as shown by broken lines, includes these readings.

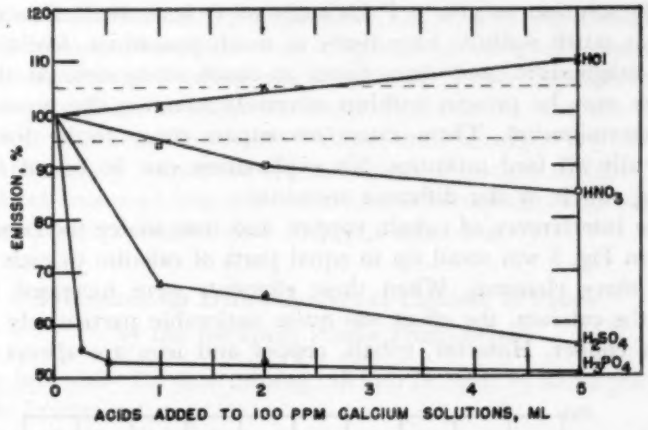

Fig. 4. Effect of Added Acids to Calcium Solutions—Measurements were made with the use of a filter. Conditions: Solution containing 100 p.p.m. calcium used to set instrument at 100% emission; Oxygen = 15 psi; Acetylene = 5 psi; Slit = 0.3; Red phototube; Wavelength = 622 $m\mu$; Flame background = 4.0% emission; Didymium light filter used.

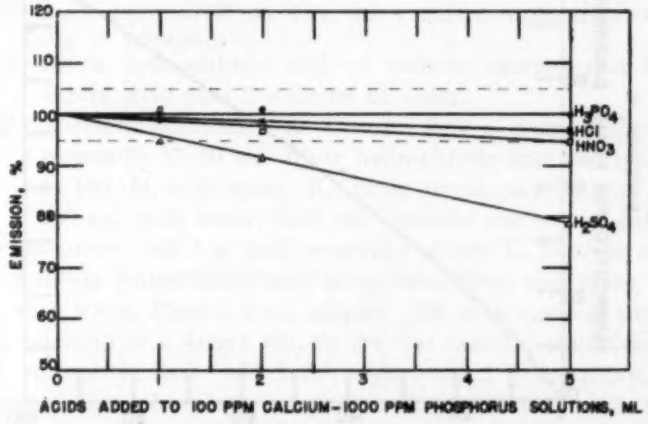

Fig. 5. Effect of Added Acids to Combined Calcium and Phosphorus Solutions—Measurements were made with the use of a filter. Conditions: Solution containing 100 p.p.m. calcium plus 1000 p.p.m. phosphorus used to set instrument at 100% emission; Oxygen = 15 psi; Acetylene = 5 psi; Slit = 0.5; Red phototube; Wavelength = 622$m\mu$; Flame background = 18.0% emission; Didymium light filter used.

The data in Fig. 3 were obtained by using solutions containing 100 ppm. of calcium plus 1000 ppm. of phosphorus to which were added increments of the other elements. In these trials, the calcium-phosphorus solution was used to set the instrument at 100% emission and the didymium filter also was used. The results indicate that when sufficient excess phosphorus (as phosphate) is present in a calcium solution to give a P/Ca ratio of at least 10/1, about seven times as much sodium, four times as much potassium, five times as much magnesium, and three times as much manganese as there is calcium may be present without adversely affecting the accuracy of the determination. These ratios encompass those to be found in practically all feed mixtures. No explanation can be found for the varying effects of the different elements.

The interference of cobalt, copper, and iron under the conditions given in Fig. 3 was small up to equal parts of calcium to each of the above three elements. When these elements were increased to ten times the calcium, the effect was quite noticeable particularly in the case of copper. However, cobalt, copper and iron are always found

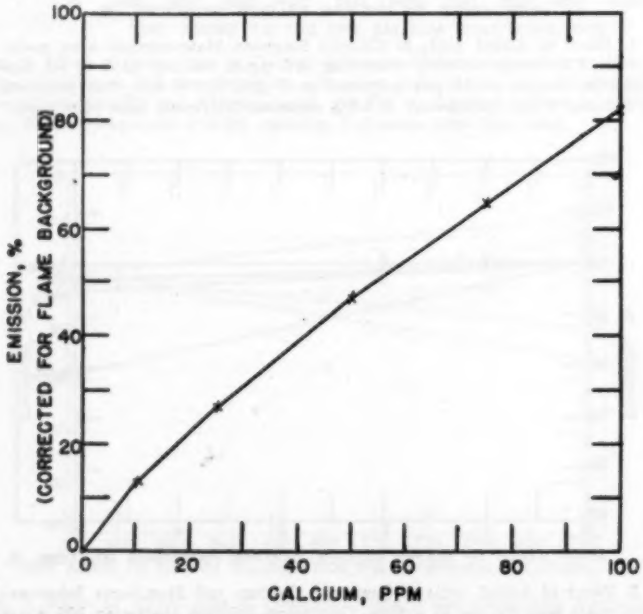

Fig. 6. Standard Curve for Calcium Flame Measurements. Conditions; All solutions contain 1000 p.p.m. phosphorus; Oxygen = 15 psi; Acetylene = 5 psi; Slit = 0.5; Red phototube; Wavelength = 622 $m\mu$; Didymium light filter used; Flame background = 18.0% emission.

in feed ash in much lesser quantities than is calcium, and their interference with calcium flame emission would be small at 622 mμ.

To select the most suitable acid for dissolving the feed ash, 1, 2, and 5 ml. of concentrated hydrochloric, nitric, sulfuric and phosphoric acids respectively were added to the different series of plain calcium solutions shown in Fig. 4, and to solutions of calcium containing added ammonium phosphate in Fig. 5. The depressing effect of phosphorus as phosphoric acid is again seen. Although phosphoric acid was used as the source of phosphorus in this experiment, ammonium phosphate solution was more desirable because the depressing effect was more constant. Sulfuric acid changes the emission spectra of calcium because of the formation of calcium sulfate.

Hydrochloric acid was selected as the most suitable acid medium, a finding which coincides with that of Parks, *et al.* (4).

Procedure for Determination of Calcium in Feeds by the Beckman Flame Photometer

The procedure for determining calcium in feeds by flame photometry is as follows:

Apparatus. Beckman flame photometer.

Reagents.

(1) Ammonium phosphate solution – 40 g. diammonium hydrogen phosphate, reagent grade, dissolved in water and made up to 100 ml. One ml. contains approximately 100 mg. of phosphorus.

(2) Dilute hydrochloric acid – 1 volume concentrated hydrochloric acid plus 2 volumes of water.

(3) Standard Solutions – A. Dissolve 2.50 g. pure calcium carbonate in 15–20 ml. dilute hydrochloric acid and make up to 100 ml. with water. B. Dilute 10 ml. of solution "A" to 100 ml. with water. Each ml. contains one mg. of calcium.

Determination. Ash 5 g. feed overnight at 600°C. Dissolve ash in 15 ml. of dilute hydrochloric acid using heat, filter and make up to 100 ml. with water. Place a 5 ml. aliquot (for feeds ranging from 1.0 to 4.0% calcium) or a larger aliquot for low calcium materials, in a 100 ml. volumetric flask, add 1 ml. concentrated hydrochloric acid, 1 ml. ammonium phosphate solution, make to volume with water, and mix.

Adjust the Beckman spectrophotometer[3] by setting selector switch at 0.1, phototube load resistor at position 2 (this uses the 10,000 meg.

[3] These specific adjustments for slit width, gas pressures, etc., may vary slightly depending on the individual Beckman used.

ohm resistor provided for flame measurements), wavelength at 622 mμ, slit at 0.5, transmission scale at 100%, adjust dark current knob to zero galvanometer needle, insert didymium filter, and use "red" phototube. Open phototube shutter switch for emission readings.

Adjust flame unit by setting oxygen pressure at 15 psi. and acetylene pressure at 5 psi. Atomize highest standard calcium-phosphorus solution of 100 ppm. calcium and bring the galvanometer needle to zero by adjusting the sensitivity knob. Thus, the instrument is set at 100% emission using the calcium standard 100 ppm. (see standard curve). Atomize a blank solution containing only water plus 1 ml. concentrated hydrochloric acid and 1 ml. ammonium phosphate solution per 100 ml. Read per cent emission; – this is the "flame background" and must be substracted from all readings for calcium solutions. Atomize unknown solutions, record per cent emission, and subtract the flame background reading. Apply to standard curve.

Standard Curve. Place aliquots of standard calcium solution "B" in a series of 100 ml. volumetric flasks. To each flask add 1 ml. concentrated hydrochloric acid plus 1 ml. ammonium phosphate solution and make up to volume with water as described in "Determination". Atomize and take readings.

Example of readings for curve:

Aliquots of Solution "B" 100 ml.	*p.p.m. Calcium*	*% Emission*	*Corrected for Flame Background*
10 ml. (+ acid & phosphate)	100	100.0	82.0
7.5 ml. (+ acid & phosphate)	75	83.0	65.0
5.0 ml. (+ acid & phosphate)	50	65.0	47.0
2.5 ml. (+ acid & phosphate)	25	45.0	27.0
1.0 ml. (+ acid & phosphate)	10	31.0	13.0
0 ml. (+acid & phosphate)	0	18.0 = Flame Background	

The readings corrected for flame background are plotted in Fig. 6.

Discussion and Conclusions

The procedure for the determination of calicum by flame photometry has been used satisfactorily for feed control purposes. As many as forty unknown solutions have been analyzed for calcium in less than 15 minutes. The cost per determination is much less than for chemical methods both as to materials used and time consumed.

Table 1 indicates that the accuracy closely parallels that of the oxalate method for calcium (1). Replicate determinations check each other as well or better than by the chemical procedure.

TABLE I

CALCIUM CONTENT OF FEEDS AS DETERMINED BY FLAME PHOTOMETRY AND BY CHEMICAL ANALYSIS

Sample	Per cent Calcium	
	Flame	Chemical
	%	%
Turkey Feed	2.28	2.21[1]
Poultry Feed	1.84	1.86[1]
Hog Feed	2.36	2.50[1]
Dairy Feed	1.60	1.51[1]
Soybean Oil Meal	0.29	0.27
Cottonseed Meal	0.11	0.12
Wheat Middlings	0.09	0.12

[1] Average collaborative results from eight laboratories.

The flame procedure should expedite this type of analysis in any feed or nutrition laboratory.

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AN EVALUATION OF FOUR FEED SAMPLING DEVICES[1]

P. R. Stout, J. W. Money, and M. P. Wineberg

ABSTRACT

The Seedburo Gamet divider, two double-tube slotted probes of different diameters, and insertion of a 1 qt. can into the feed stream from the packing spout all gave comparable samples, as judged by protein analysis, from a poultry mash and a molasses dairy feed. The two probes gave high results when used to sample a dairy feed consisting of pelleted low-protein materials and finely-divided high-protein meals.

Particle-size measurements on samples taken from a known mixture of granular materials showed that the two probes discriminate in favor of finely-divided materials. The standard single-tube trier probably behaves similarly.

The first requirement for a correct evaluation of any material is to obtain a representative sample. It is therefore surprising that the literature contains so little information on the sampling of certain commercially important materials, particularly mixed feeds.

Miles and Quackenbush (8) have noted that many current sampling methods were developed in the absence of much basic information concerning factors which affect representativeness and precision. Sampling theory has been comprehensively treated by Deming (5). Manning (7) and Buslik (4) have contributed papers on the mixing and sampling of multi-sized materials, but there is very little published information on the performance of several commonly used feed sampling devices.

Commercial feed mixtures present difficult sampling problems because of their wide ranges of particle sizes and because of their physical characteristics. They may be free-flowing granular mixtures or the particles may cohere, as is the case with very fine meals or flours, or grains mixed with molasses.

It is important to distinguish among the three kinds of variability encountered in an experiment concerned with sampling methods: (1) product variability, (2) sampling variability, and (3) analytical variability.

Product variability is independent of sampling methods and of analytical methods. The true composition of a feed, for example,

[1] Manuscript received June 26, 1952. Presented at the Annual Meeting, May 1952. Contribution from the Research Laboratories of The Quaker Oats Co., Chicago, Ill.

will vary from sack to sack or batch to batch. It will depend on mixing time and equipment, fluctuations in ingredient quality, feeder rate variations, etc. The manufacturer is very much interested in product variability but before he can measure it he must precisely establish sampling and analytical variabilities.

Sampling variability arises in the process of collecting samples. It is often referred to as the precision of the sampling method. It may be expected to be different for different sampling methods and devices.

Analytical variability refers to differences in results when separate portions of the same sample are analyzed. These differences are inherent in the nature of chemical operations and measurement techniques of all kinds. Analytical variability must be made insignificantly small or be precisely established and controlled by a suitable sampling plan.

It is desirable to measure both product and sampling variability so that their magnitudes may be compared. The two types of variability may be separated through the use of appropriate experimental or mathematical techniques (6). Sampling errors may be isolated by using several different devices for sampling each of a number of subdivisions of a lot, or by sampling a calibrated standard. If the sampling variability is considerably less than the product variability, then the sampling method is suitable for use with that product. If sampling variability is greater than the product variability, the sampling method is inadequate for use with that product.

Usually the most precise sampling method is the most desirable. However, it is entirely possible for a particular sampling method to be precise yet very inaccurate. In such a case results on different samples would check well, yet be far from the truth. Before such a sampling method could be put to practical use, it would be necessary to measure the sampling bias or amount of inaccuracy, and then apply this figure as a calibration or correction when using this sampling method.

The present study is an evaluation of four sampling techniques on three types of feed.

Experimental

This study originally included two types of probes, two types of mechanical dividers, and a hand sampling technique. However, aggregates of one of the feeds studied clogged the chutes in one of the dividers, so that a sample could not be obtained. This divider was

omitted because versatility was considered an essential characteristic of a feed sampling device.

Three kinds of feed were selected: (1) a typical poultry mash, (2) a dairy feed consisting of a pelleted premix (3/16 in. pellets) and crimped grains over which 10% of molasses had been sprayed, and (3) a dairy feed consisting of a premix pelleted into 3/16 in. pellets, soybean meal, distillers grains, and gluten feed. Particle size data for these three feeds were as follows:

	No. 1	*No. 2*	*No. 3*
% over U.S. No. 6	0.0	41.0	37.6
% over U.S. No. 8	1.0	40.1	0.1
% over U.S. No. 12	4.3	4.1	3.3
% over U.S. No. 16	7.2	8.0	12.9
Thru U.S. No. 16	87.4	6.8	46.1

Ten consecutive 100-lb. bags of each of the three feeds were removed from the packing line after quart samples had been taken by inserting a can into the stream from packing spout to bag. The bags were probed diagonally with double-tube compartmented probes. One probe was 0.625 in. inside diameter, 36 in. long, and had 2.50 in. by 0.625 in. slots. The second probe was 1.125 in. inside diameter, 36 in. long, and had 3.50 in. by 0.875 in. slots. Each bag was probed a sufficient number of times to give a quart sample. The remaining feed (about 97 lbs.) was cut on a Gamet Divider six times to give a 1.5 lb. sample. A special hopper (about 120 lbs. capacity) was fitted to the Gamet Divider since the regular hopper was too small for our purpose.

Samples were ground to pass a 2 mm. sieve, mixed and quartered twice to give 6 oz. samples which were ground to pass a 1 mm. sieve. Duplicate Kjeldahl nitrogen analyses were made on each sample. The data are presented in Table I.

The results of individual determinations yielded an estimate of 0.13% protein for the standard deviation of the chemical analysis.

With Feeds No. 1 and No. 2, the four sampling methods agreed well. With Feed No. 3, which consisted of a mixture of pelleted low-protein materials and finely-divided high-protein meals, the two probes were, on the average, more than one percent higher in protein than the can and divider samples. In a separate study, the divider did not discriminate in favor of high-protein components. The data indicate, therefore, that the two probes discriminate in favor of high-protein components.

If probes discriminate in favor of the finer particle sizes, then

TABLE I

PERCENT PROTEIN IN SAMPLES TAKEN FROM THREE FEEDS BY FOUR METHODS
(averages of duplicate protein (Nx6.25) determinations

Bag No.	Feed No. 1 (Poultry Mash)				Feed No. 2 (Dairy Feed)				Feed No. 3 (Dairy Feed)			
	Can Sample	Probes		Gamet Divider	Can Sample	Probes		Gamet Divider	Can Sample	Probes		Gamet Divider
		No. 1	No. 2			No. 1	No. 2			No. 1	No. 2	
1	20.6	21.2	20.7	20.7	20.3	20.5	20.5	20.6	32.6	34.3	33.9	32.7
2	20.8	20.8	20.5	20.5	19.9	20.2	19.9	19.9	32.2	34.4	34.0	32.2
3	20.8	20.4	20.7	20.6	20.3	20.3	19.9	20.0	31.7	33.3	33.4	32.4
4	20.4	20.6	20.4	20.7	19.9	19.5	19.7	19.7	32.5	34.5	33.7	32.5
5	20.3	20.4	20.4	20.4	20.0	20.0	20.1	20.7	33.3	33.7	34.2	33.0
6	21.1	20.5	21.0	20.5	20.2	20.0	20.7	20.1	32.4	34.7	33.9	32.3
7	20.5	21.2	20.9	20.7	19.8	20.5	20.2	20.0	32.2	33.3	33.3	32.1
8	20.7	20.9	20.9	20.9	19.5	19.8	20.1	20.3	31.9	34.0	32.9	32.3
9	20.9	21.0	20.9	20.8	19.8	19.8	19.5	20.0	31.2	34.2	33.1	31.9
10	20.7	20.9	20.8	20.8	19.8	20.3	20.5	20.2	32.5	34.4	33.9	32.9
Av.	20.7	20.8	20.7	20.6	20.0	20.1	20.1	20.2	32.2	34.1	33.6	32.4

repeated probing of a sack should increase the concentration of larger particle sizes in the material remaining in the sack. A second experiment, in which 21 probe samples were taken from a single sack of Feed No. 3 proved to be a very inefficient method for demonstrating probe discrimination. The regression of the sample protein on sample number had a coefficient of -0.043 ± 0.06. This result is in the region which would be expected if the probes give results 1 or 2% high, but it was not possible to decide whether the regression coefficient was actually different from zero. Many more samples would be required to get a very exact value for the regression coefficient. Better methods are available for studying the behavior of sampling probes.

The experimental procedure was to mix weighed amounts of carefully screened grains or grain products in a drum mixer of 100 lbs. capacity, sample the mixture through a hole in the drum, sieve the sample, calculate the percentages of the various sizes, return the sample to the drum, remix, resample, etc.

The experimentally determined percentages of the various sizes, when compared with the known composition of the mixture, measure the accuracy of the sampling device with respect to the various particle sizes. The same data provide estimates of sampling precision.

The particular mixture from which the data of Table II were obtained consisted of:

(1) Whole corn over a 20/64 in. R.H.P.
(2) Whole corn thru 18/64 in. R.H.P. – over 16/64 in. R.H.P.
(3) Whole wheat over U.S. Sieve No. 8
(4) Corn grits thru U.S. No. 16 – over U.S. No. 20

Intermediate sizes (e.g. thru 20/64 in. – over 18/64 in.) were purposely omitted to reduce errors arising from abrasion and breakage during mixing and sampling. The next finer sieve sizes were used for sieving the samples further to reduce such errors.

TABLE II

PARTICLE SIZE DATA FOR SAMPLES TAKEN FROM "STANDARD" MIX
(Averages for 15 Samples)

	Over 18/64 in.	Over 14/64 in.	Over U.S. No. 10	Thru U.S. No. 10
	%	%	%	%
As mixed	14.0	14.0	18.7	53.3
Probe No. 1	7.4	7.6	18.8	66.3
Probe No. 2	9.8	9.7	18.6	61.9
Gamet Divider	13.8	13.9	18.9	53.4

The probe samples from this mixture were grossly inaccurate for three of the four particle sizes. The percentage of fine material in the probe samples was too high, and the percentage of coarse material was too low. Probe No. 1 which had the narrower slots, showed the greater discrimination in favor of finely-divided material. The probe inaccuracy which appears in Table I as a discrimination in favor of high-protein materials is therefore actually a discrimination in favor of small-size particles. The standard trier (a single-tube probe used by many inspectors to sample feeds and fertilizers) was not investigated in this study, but previously reported studies (1, 2, 3) showed no significant differences in fertilizer samples obtained with single and double tube samplers.

A large fraction of dairy feed production consists of granular mixtures in which pellets are used as carriers for trace minerals, vitamins, drugs, antibiotics and light-sensitive materials. Small variations in the percentages of these substances greatly affect feed performance. Thus the composition of the feed must be accurately known, which means that sampling and analytical errors must either be insignificant or be precisely established. There is the additional reason, of importance to both the manufacturer and inspector, that tag claims must be met.

The manufacturer can use a non-discriminating sampling device on the mixing line or at the packer. For example, the entire stream might be temporarily diverted to a disc (Gamet) or riffle type divider. Since these devices merely halve the stream, they would have to be connected in cascade or provision be made to return half the stream to the divider until the sample was reduced to convenient size. The problem is more difficult for the inspector. He uses a probe because it is a convenient and economical way of sampling feed or fertilizer without damaging the lot units beyond repair. However, free-flowing heterogeneous materials, such as those used in the present study, are not accurately sampled by means of probes. The inspector is therefore faced with the necessity of designing a probe which will not discriminate, or of establishing precisely the degree of discrimination occurring with the various multi-sized granular mixtures which must be sampled. In addition, the inspector is ordinarily not in a position to sample from the entire production lot. His results may therefore differ from those of the manufacturer for this reason also (5, page 21; 9).

Our data indicate that the observed discrimination depends on particle size and probe diameter. In order to establish the nature of the dependence, additional experiments would be required, involv-

ing more particle sizes and wider ranges of sizes. It would be desirable also to study the precision of non-discriminating sampling devices when used on feed materials, as a step toward development of rational sampling procedures which are adequately accurate and precise.

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A NEW COLOR METHOD FOR DIFFERENTIATING BRAN AND GERM PARTICLES FROM INSECT FRAGMENTS IN FLOUR[1,2]

R. A. LARKIN, M. M. MACMASTERS, AND C. E. RIST[3]

ABSTRACT

A new method is described for the differentiation of bran and germ particles from insect and larval fragments in wheat flour. It consists essentially of suspending the flour in 0.6 *N* sodium hydroxide which largely disperses the starch and protein, partially neutralizing with hydrochloric acid, filtering through a wire screen, submerging the screen and retained particles in 0.025% Crystal Violet solution for 15 minutes and observing the fragments in the solution by transmitted light. The bran and germ particles are stained deep violet, while the insect and larval fragments appear bright orange red. Insect and larval fragments so small as to be identified by structural details only with difficulty are easily distinguished by this method.

The number, size, and structural composition of bran and germ particles in experimentally milled flours became a matter for study in an investigation of the structural differences correlated with milling quality of some Pacific Northwest wheat varieties. A method was devel-

[1] Manuscript received June 9, 1952.
[2] Presented at the Annual Meeting of the American Association of Cereal Chemists, Dallas, Texas, April 20-24, 1952.
[3] Northern Regional Research Laboratory, Peoria, Ill. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

oped for the rapid identification of bran and germ particles in flour (8). The question arose as to whether or not insect and larval fragments, if present, would stain like bran and germ and thus be confused with the latter in the sample. Insects added to the test samples were not stained. They appeared red in the stain solution, but when the suspension was filtered they returned to their original brown color and were difficult to distinguish. It was evident that a slight modification of the technique which had been developed for detecting bran and germ fragments would provide a method for the rapid differentiation of bran and germ particles from insect and larval fragments.

Present methods for detecting insect and larval fragments in flour include flotation of the fragments from the bulk of the flour and their subsequent identification by morphological characteristics. Difficulties have been encountered in the separation of the fragments by flotation (10). In addition, many discrepancies in determinations have been attributed to the difficulty of identifying the insect and larval fragments (6). Several investigators (2, 4, 5) have described the identifying characteristics of the various morphological parts of flour beetles, but as Walker (9) has pointed out, the fragments vary in size from large to extremely small. The smaller the fragments, the more difficult they are to identify by their morphological characteristics. It is the small insect and larval fragments which are easily confused with small bran and germ particles. Gier, *et al.* (3) suggested a differential staining procedure, using Azure 1, which makes it possible to identify the insect fragments by color. In a study of three differential stains, Azure 1, Methylene Blue, and Methyl Green, the members of the Sanitation Methods Committee of the American Association of Cereal Chemists preferred Methyl Green (7). The staining methods were considered, however, to be inferior to methods depending upon morphological identification of the fragments. Staining when used has always been applied to fragments mechanically separated from the flour (1).

The following method was developed by which bran and germ particles can be quickly differentiated from insect and larval fragments without recourse to morphological identification of the latter.

Materials and Method

Adult flour beetle (*Tribolium* spp.) fragments, which served as reference material, were mixed with flours to form samples which were used during the development of the method. Later, National Check Sample No. E-6 flour of the Sanitation Methods Committee of

the American Association of Cereal Chemists, 1951 series, was used to check the accuracy of the method developed.

Equipment used in the method is as follows: 4-l. beaker, stirring rod, 500 and 100 ml. graduated glass cylinders, 9 cm. coarse porosity fritted glass funnel, suction flask, 9 cm. disc of 200- mesh wire screen with 74 μ diameter openings, 9 cm. petri dish, low-power binocular dissecting microscope, and a fluorescent desk lamp.

The method, which can be easily completed in 25 minutes, is as follows: Place 15 g. of flour in 55 ml. of 95% ethyl alcohol in a 4-l. beaker. Stir until there are no aggregates of flour. Next, add in succession mixing well at the end of each addition 500 ml. of distilled water, 500 ml. of 5% (1.25 *N*) sodium hydroxide, 1-l. of distilled water, and 40 ml. of concentrated (12 *N*) hydrochloric acid at room temperature. Filter with suction through a 9 cm. coarse-porosity fritted glass funnel, with a tight fitting disc of 200-mesh wire screen on top of the fritted glass disc. After filtration, place the wire disc in a petri dish and cover the screen with 0.025% aqueous Crystal Violet (C. I. No. 681) solution – usually 40 to 50 ml. of the solution is adequate. Let stand for 15 minutes, then place the petri dish and contents under the dissecting microscope. Observe the insect fragments by using transmitted light from a source such as a fluorescent desk lamp, and the concave substage mirror.

With this method, an average of 41.3 (range, 23 to 72) insect and larval fragments was found in 9 samples of 15 g. each, equivalent to 137 fragments in a 50 g. sample. The National Check Sample Committee reported an average of 18.64 (range, app. 1 to 94) insect fragments and 5.19 (range, app. 0 to 30) larval fragments in 50 g. samples of the same flour. The sodium hydroxide–Crystal Violet method does not distinguish between insect and larval fragments.

Discussion

Insect fragments appear bright orange-red, while the bran particles are stained deep violet. Larval fragments do not appear as bright as the adult insect fragments. Only the exoskeleton of either larva or adult appears orange-red. The internal parts are stained the same color, violet, as the bran particles. The red coloration of the exoskeleton is not a staining phenomon, since the insect fragments show no red color when removed from the stain. Therefore, the observations must be made while the fragments are in the stain solution.

It is important that steps 3, 4, 5, and 6 be completed in rapid succession. If the alkali is permitted to act for 10 minutes or longer, the

bran particles appear dull red instead of violet and are easily confused with the insect particles. Also, the color of the insect fragments appears less brilliant. The morphological characteristics, however, are not altered by the treatment, and can be used for confirmation of the identity of insect fragments, if desired.

During the present study, satisfactory illumination was obtained from two 15-watt fluorescent white tubes; any incandescent desk lamp could, however, be used. A microscope lamp is too strong a source of light and causes the entire field to appear red.

With proper cleaning, the wire screen and fritted glass funnel can be used repeatedly. The former was satisfactorily cleaned by dipping in chromic acid cleaning solution and thoroughly washing in tap water, alternately, three times each. Adequate cleaning of the funnel was attained by submerging it in the cleaning solution for 20 to 30 minutes. A funnel with a removable fritted glass plate would undoubtedly be easier to clean than one with a permanent disc which was used in this study.

The high fragment count obtained by the present method in comparison with the average reported by the National Check Sample Committee may be attributed to two causes: (a) Greater recovery of the small fragments, which of course is dependent upon the mesh of the screen used. A 100-mesh screen would not retain the fragments smaller than 149 μ in diameter, while a 325-mesh screen would retain the fragments as small as 44 μ in diameter. The refinement of the method as practiced is dependent upon the mesh size of the screen selected. (b) Easier differentiation of fragments. The present method facilitates the differentiation of small insect fragments from bran since only color, not morphology, need be distinguished. Pieces of thorax, for example, which have only three or four pits and are very difficult to identify by their morphology, are easily differentiated by the present method. Some of the smaller fragments, even with too few morphological characteristics for identification can be sharply differentiated by their color in the Crystal Violet solution.

Since the insect and larval fragments are easily distinguished from bran and germ particles by color alone, highly trained personnel are not required to conduct the test. Workers using this method for the first time check each other to within one or two fragments. Adequate sampling methods remain a problem.

Acknowledgments

The authors are indebted to Mr. F. R. Earle of this Laboratory for assistance in obtaining samples for study, to Mr. James Doty who supplied a flour sample

used by the National Check Sample Committee of the American Association of Cereal Chemists, and to Mr. C. S. Newell who provided data on the insect contamination found in the sample by Committee members.

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OBSERVATIONS ON A SIXTY-FOUR YEAR OLD WHEAT SAMPLE[1]

R. H. Harris[2] and H. L. Walster[3]

ABSTRACT

A sample of Power, a selection from Fife, grown in North Dakota in 1888 and which had been kept in a tightly stoppered glass bottle was found to be entirely lacking in viability. The milling quality was below normal, while baking performance was extremely poor as compared with samples of the same variety grown in the experimental plots at Fargo in recent years. There was some indication that the protein had become partially coagulated. Proteolytic activity was markedly below that of the other comparative samples.

The effect of age on the viability and quality of wheat is of interest to cereal workers. Recent experiments at Fort Collins, Colorado, (2) show that breeding stocks may be stored from 15 to 20 years without replanting to maintain viability. Marquis, Kanred and Kubanka

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wheats averaged 10.4% germination after 27 years' storage, while barley showed over 25% viability after 28 years. Rosen rye had the lowest viability, 5.6%, after 15 years. It was pointed out, however, that most seeds decline sharply in viability following 10 years of storage. Some properties of ancient Egyptian "mummy" wheat harvested over 3000 years ago have also been discussed (7). The protein content was higher than would be expected, possibly because of starch disintegration and loss of dry matter due to oxidation of the degradation products.

In the present paper some observations are reported on the viability, protein content, enzymic activity, and milling and baking quality of a sample of hard red spring wheat grown in 1888 in comparison with corresponding data for the same variety grown in recent years.

Materials and Methods

Power, a variety of hard red spring wheat which was grown at Arvilla, a small community west of Grand Forks, North Dakota in 1888 and at the Fargo plots in 1949, 1950, and 1951 was used. It is a late maturing variety, and therefore more subject to heat, drought, and rust injury than early maturing varieties. The sample (about 500 g.) had been kept in a tightly stoppered glass bottle. It was bright and sound in appearance and showed no damage.

Because of the limited quantity of wheat, micro milling and baking methods were employed (4, 8). Viability tests were made by the North Dakota State Seed Department. Nitrogen was determined by the Kjeldahl-Wilfarth-Gunning method. Moisture, ash, and diastatic and proteolytic activity were ascertained by accepted procedures (1, 3). Mixograms were obtained and soluble nitrogen found by techniques already described (5, 6).

Results and Discussion

Table I shows the viability and commercial grade of the four samples together with wheat protein content and milling data. The old wheat kernels appeared to be hard and brittle, the germ portion often breaking away during scouring. They were entirely lacking in viability, but in other respects compared favorably with the more recent samples, with about 2% higher protein content. The old wheat was particularly low in yield of long patent flour, while the low grade flour was approximately double that of the other samples. No marked difficulty in milling, however, was reported by the miller.

TABLE I

VIABILITY, GRADING, AND MILLING DATA FOR THE FOUR SAMPLES OF POWER WHEAT

Crop Year	Viability	Test Weight	Unofficial Grade	Approx. Vitreous Kernels	Wheat Protein	Flour Yield		
						Long Patent	Low Grade	Total
	%	*lbs/bu*		%	%	%	%	%
1949	95	61.5	1 HDNS	85	11.7	66.4	3.5	69.9
1950	92	59.0	1 DNS	90	11.8	64.7	2.3	67.0
1951	97	62.0	1 HDNS	80	11.7	65.9	3.5	69.4
1888	0	58.5	1 DNS	80	13.8	58.3	6.8	65.1

Note: All quantitative data on a 14.0% moisture basis.

TABLE II

ASH CONTENT AND BAKING QUALITY OF THE FLOUR FROM THE FOUR SAMPLES OF POWER WHEAT

Crop Year	Ash	Absorption	Loaf Volume	Grain and Texture[1]	Crumb Color[2]	Crust[3]	Symmetry[4]
	%	%	*cc.*				
1949	0.47	59.6	185	7.5	7.5	S	4.5 O
1950	0.35	59.6	190	7.5	7.5	S	4.5 O
1951	0.51	59.6	185	7.0	6.5	S	4.0 O
1888	0.59	75.6	95	4.0 C&O	1.0 Br.	P	0.5 O

[1] Grain and Texture: O = open; C = coarse.....Perfect Score 10.0
[2] Crumb Color: Br. = brown.....Perfect Score 10.0
[3] Crust: S = satisfactory; P = pale.
[4] Symmetry: O = overoxidized.....Perfect Score 5.0

Table II provides information on the ash content and baking quality of the flours. There was marked disparity in ash among the flours, with the old wheat having the highest value. However, the difference between it and two of the others was less than that between some of the satisfactory flours. The absorption of the flour from the old sample was extraordinarily high being 16% above the three Fargo wheat flours. The loaf was one of the worst ever baked in this laboratory, with only one-half the volume of the other flours. The dough felt heavy and "lifeless," but did not slacken during fermentation. All loaf properties were very poor, showing that the wheat was totally unfit for baking.

Figure 1 represents the appearance of the loaves. The inferior quality of the old wheat loaf is very apparent; it was not flat, but simply failed to rise either during fermentation or in the oven.

Figure 2 shows the mixograms obtained from the flours. The curves for the Fargo grown samples denote strong, satisfactory flours. The curve for the flour from the 1888 wheat, however, is decidedly unusual,

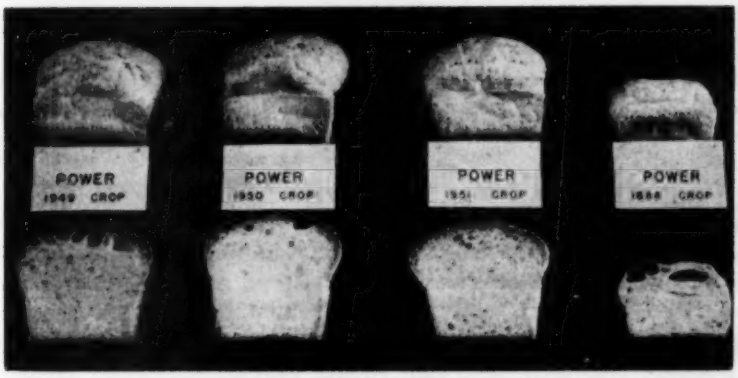

Fig. 1. Appearance of loaves baked from flours of normal and 64 year-old wheat.

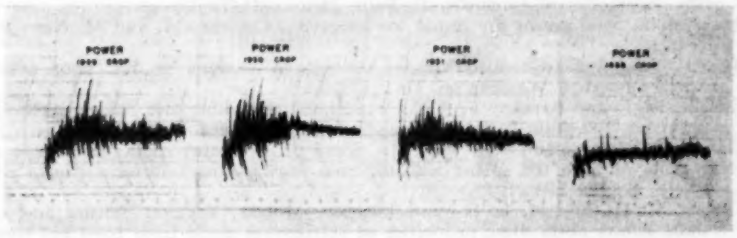

Fig. 2. Mixing characteristics of flours from normal and 64 year-old-wheat.

with little dough development, and a slow gradual rise. This appearance, coupled with the abnormally high absorption, aroused suspicion that the protein had become partially coagulated, and to obtain further information on this point the four flours were extracted with 0.1 *N* acetic acid for six hours in a rotary shaker (5), with the following results:

Crop Year	Nitrogen extracted by shaking	mg^{N}ml % of total N.
1949	0.97	75.4
1950	1.10	82.5
1951	0.94	73.1
1888	1.02	66.0

When calculated as % of total nitrogen the data indicate less solubility for the old wheat flour, and lend support to the hypothesis that some coagulation had occurred.

Impairment of enzymatic activity had apparently occurred, particularly of the proteases, as shown in Table III.

TABLE III

PROTEIN CONTENT AND ENZYMATIC ACTIVITY OF THE FLOURS[1]

Sample	Flour Protein	Diastatic Activity	Proteolytic Activity
	%	*mg. maltose 10 g. flour*	*mg. sol. N. g. protein*
Composite[2]	10.5	128.0	13.7
1888 Crop	12.4	110.5	4.5

[1] Expressed on 14.0% moisture basis.
[2] Composited from the 1949, 1950, and 1951 flour samples.

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COMMUNICATION TO THE EDITOR

The Return of Birefringence to Gelatinized Starch Granules

Dear Sir:

Starch granules exhibit birefringence before gelatinization. When viewed between crossed Nicol prisms, each shows a dark cross which divides the granule into four brilliant segments.

When the granules are heated in water to a sufficiently high temperature, their birefringence is lost. Above a certain temperature, depending upon the kind of starch, the size of the granule, and probably other unidentified factors, the cross gradually enlarges at the intersection of its arms, i.e., at the hilum of the granule. The bright segments within the four arms of the cross consequently decrease in size and eventually disappear entirely. They usually persist longest at the periphery of the granule. When all birefringence has been lost, the granule is said to be gelatinized. There is usually a temperature range of several degrees from the beginning of gelatinization, when the cross begins to enlarge at the hilum, to completion, when the entire granule has lost birefringence. Upon further increase in temperature, the granules swell to twice or more their original size.

It was observed that birefringence reappeared in the outer portion of gelatinized granules upon the addition of iodine solution. This occurred with starch which had been heated in water to 10° or 15°C. above the temperature at which birefringence was completely lost (Fig. 1). The solution of the reagent was added at the side of a mount of the starch in water, so that a concentration gradient was set up. It was thus possible to observe the effect of the reagent over a range of concentration. The phenomenon occurred in barley, corn, oat, rice, rye, sorghum, wheat, arrowroot, canna, potato, sweetpotato, and tapioca starches. It was not exhibited by waxy corn and glutinous rice starches.

The birefringence observed after the addition of iodine solution to partially swollen starch was fainter in root starches than in cereal starches. Cereal starches which had been methanol-extracted regained weak birefringence, like the root starches. A sorghum starch which had been treated with $N/32$ sodium hydroxide solution during processing exhibited no return of birefringence.

Occasionally the phenomenon was observed in starches that had

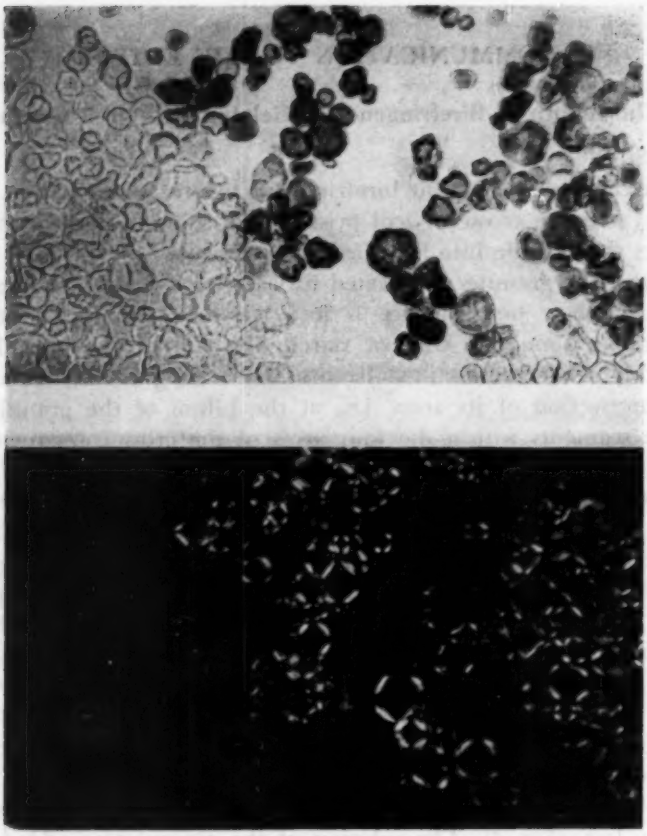

Fig. 1. Corn starch in water heated to 85° C. (Top) The dark granules have been stained by iodine solution added at one side of the cover slip. (Bottom) Same field between crossed Nicol prisms, showing return of birefringence to stained granules. Magnification 200X.

been heated as high as 30°C. above their gelatinization temperature. It was brightest and most usually observed in granules heated to not more than 10°C. above their gelatinization temperature.

The effect of absolute ethanol, sodium oleate, oleic acid, butanol, and monoethanolamine, respectively, was tested by adding each to an aqueous starch suspension. That of aqueous solutions of benzopurpurin, congocorinth, methyl orange, methyl violet, orange G, pinacyanole, and Sudan II was determined by the technique described for the iodine solution. Only ethanol and pinacyanole caused return of birefringence to the outer portion of the gelatinized granules. About five minutes was required for the phenomenon to appear after the

addition of pinacyanole, during which time the color of the granule changed from rose to blue.

Neither iodine solution nor pinacyanole appreciably affected the granule size. Ethanol, however, caused a considerable shrinkage of the granules concomitant with the reappearance of birefringence in the outer portion. Upon the addition of water at room temperature, the granules regained their swollen size and lost all birefringence.

These observations suggest that the disorganization of starch granule structure which occurs during gelatinization is incomplete and that some organization is persistent even when birefringence of the granule is no longer microscopically observed.

The experimental work cited was largely done by C. W. Bice, who also took the accompanying photomicrographs of material prepared by him.

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July 1, 1952

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BOOK REVIEWS

The Chemistry and Technology of Food and Food Products. Edited by Morris B. Jacobs. Second edition, 1951. Volume II, pp. 835–1769; Volume III, pp. 1773–2580. Interscience Publishers, Inc., New York. Price, $15.00 per volume.

These two volumes complete this comprehensive and authoritative treatment of the many varied aspects of food technology. The first volume comprised of parts 1, 2, and 3 dealt with the fundamentals of food chemistry, unit operations and processes, and the maintenance of sanitary and quality control. The second volume consists of part 4 which is devoted to the more "descriptive" aspects of some of the more important food products, such as dairy and meat products; seafood; cereal grains and bakery products; fruits and vegetables; edible fats and oils; confectioneries; coffee, tea, and cocoa. "Descriptive" is meant to include such diverse topics as history, statistics, definitions, standards, composition, and chemistry of certain important food groups. The third volume contains parts 5 and 6. Part 5 deals with the principal methods of preserving foods, such as dehydration; refrigeration; use of hermetically sealed containers; use of microorganisms; chemical preservatives; packaging, and part 6 is closely related to part 4 inasmuch as it is concerned in the main with the production methods of the food groups considered in the latter section. In some instances, however, where the food material did not lend itself to treatment in separate chapters, the resulting single chapter was placed in part 4, if more descriptive, and in part 6, if more concerned with production.

Although it is a difficult matter to assess the relative importance of a particular food, some obvious inequalities in the amount of space allotted to the consideration of some foods are apparent. Thus, the chapters on Vegetables and Mushrooms (Chapter 27) and Fruits and Nuts (Chapter 28) comprise 377 pages or over 40% of the pages of volume II. In comparison only 34 pages in volume II and 39 pages in volume III are devoted to the subject of meat and meat products, and only five pages to such an important food item as butter. An important phase of food technology such as canning was accorded only 29 pages.

Degering in his chapter on Carbohydrates and Sugar Foods (Chapter 29) has used a rather unconventional method of presenting the structural formulae of carbohydrates which may be confusing to those accustomed to the more orthodox Haworth representation. In this same chapter the formula for galacturonic acid on p. 1635 is incorrect. Stansby in his chapter on Fish, Shellfish, and Crustacea (Chapter 22) is in error when he refers to cystine as an amino acid essential to growth. The footnotes on p. 1187 are numbered incorrectly. In general, however, for a book of this size there were surprisingly few typographical errors.

The cereal chemist will be particularly interested in those chapters on the descriptive and technological aspects of cereal grains (Geddes) and bakery products (Cathcart). These chapters are to be commended not only for their excellent coverage of the literature but also for the wealth of information which has been drawn largely from the experience of the authors and their colleagues. Many of the chapters in part 6 dealing with production methods likewise contain valuable information not readily found in the literature since much of the material is based on the personal knowledge and experience of the authors.

Since each chapter contains a detailed and comprehensive treatment of a particular food or group of food products, it is not likely that the average reader will have the time or inclination to read each and every chapter. The real value of this book will be primarily in its use as a reference source dictated by the interests of the individual reader. In this connection, it should be pointed out that the reader's quest for information will be aided considerably by the very fine subject index at the conclusion of volume III covering all three volumes. This is in addition to abridged subject indices in volumes I and II.

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The Drying and Storage of Combine Harvested Grain. Published by Muntona Ltd., Bedford, England and Edward Fison Ltd., Ipswich, England, 1952. Gratis.

This little pamphlet (35 pp.) of anonymous authorship has been published jointly by two British malting firms to mark their centennial anniversary. In contrast to the purpose and content of most publications of this type, this one contains no advertising and is simply an abbreviated but fundamentally sound discussion of the problems occurring in Britain associated with the storage, drying and handling of grain. Particular stress is laid on the difficulties arising from the use of the combine harvester in that country.

The article begins with a brief listing of the advantages and disadvantages of combines in British practice. There follows a description of these machines and recommended ways for using them. Next appears an elementary discourse, based on fundamental concepts, of those properties of grain and the factors in its environment which influence its storage properties, such as water content, air humidity and composition, temperature, mold growth, insects etc.

A following section dealing with technics for cooling and drying grain is introduced by a brief discussion of some basic principles of temperature-humidity relationships of air. The discussion of drying and conditioning technics lays special emphasis on practices recommended for farm use.

Next appears a brief discussion of grain storage structures and the operation and supervision of mechanical facilities in such structures. A short but well chosen list of references to the literature completes the pamphlet.

In spite of a few minor inconsistencies in the factual portions of the presentation, cereal chemists would be well advised to bring up to date their knowledge of grain storage problems by securing and reading this pamphlet and recommending it to elevator superintendents and grain producers.

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The Science, Raw Materials and Hygiene of Baking. By Alexander Urie and J. H. Hulse. 458 pp. MacDonald and Evans, Ltd., London, 1952. Price 30s ($4.20) plus postage.

The Science, Raw Materials and Hygiene of Baking was written for students of baking and food technology and for convenience of presentation is divided into five main parts.

In Part I, the authors undertake a discussion of matter from the chemical and physical viewpoint. The electron theory is explained and the inter-conversion of states of matter interestingly developed. There are discussions on heat, cohesion, adhesion, surface tension, etc., with a very lucid explanation of the colloidal state of matter, with particular reference to emulsions and gels. A brief discourse on the chemical structure of matter with a general review of organic chemistry is followed by an interesting discussion on the application of chemistry to the science and art of baking. Particular emphasis is placed on the stabilization of emulsions, foams, creams and gels as well as on the nature of pectin and its behaviour in jellies and jams.

Part II is written in two sections and deals with the various raw materials used in bread and cake production. The changes which take place in bread doughs during mixing and fermentation are discussed and the so-called high-ratio system of cake production is presented as well as an enlightening review of bread additives.

Part III is concerned with an extensive discussion of raw materials, their origin, nature and composition. Wheat, yeast, sugar, edible oils and fats, milk, eggs, cocoa, dried fruits, flavoring agents, and coloring matter are each considered. The structure and composition of wheat and other cereals is presented in detail along with the discussion of yeast and various leavening agents such as baking powder. In dealing with malt products, no mention is made of fungal and bacterial amylases, a subject which is receiving intense study in the United States at the present time.

Part IV is written in three sections. In the first section, the authors discuss

organisms, bacterial food poisoning, and food contamination through the use of pesticides and insecticides. The second section deals with bakery sanitation and the use of various types of detergents and soaps. The third section is devoted to the preservation of food by such methods as drying and refrigeration, the keeping quality of these food materials when properly preserved, and a brief consideration of rope and bread staling.

Part V deals with nutrition, approaching it from the standpoint of taste and smell and then developing the concept of nitrogen balance and discussing the metabolism of the various food components. The authors emphasize the nutritional value of baked products by presenting considerable statistical data on their composition.

The book is well-illustrated with 23 plates and 50 other illustrations in the form of figures, tables, charts and graphs. It is of value as an elementary treatise for students who have in mind entering the cereal field as their life's work, and should also be in the library of technologists of the baking, milling and allied industries.

Charles A. Glabau
Bakers Weekly
New York, New York

Cereal Chemistry

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for **Cereal Chemistry.**

Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from and subscriptions placed with the Managing Editor, University Farm, St. Paul 1, Minnesota. Subscription rates, $11.00 per year. Foreign postage, 50 cents extra. Single copies, $2.50; foreign, $2.60. Back issues, $3.00.

SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6:1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Titles and Footnotes. Titles should be specific, but should be kept short by deleting unnecessary words. The title footnote shows "Manuscript received . . ." and the name and address of the author's institution. Author footnotes, showing position and connections, are desirable although not obligatory.

Abstract. A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions, and should contain, largely by inference, adequate information on the scope and design of the investigation.

Literature. In general, only recent papers need be listed, and these can often be cited more advantageously throughout the text than in the introduction. Long introductory reviews should be avoided, especially when a recent review in another paper or in a monograph can be cited instead.

References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also. Abbreviations for the names of journals follow the list given in *Chemical Abstracts* **40:** I–CCIX. 1946.

Organization. The standard organization involves main sections for abstract, introduction, materials, methods, results, discussion, acknowledgments, and literature cited. Alternately, a group of related studies, each made with different materials or methods, may require a separate section for each study, with subsections for materials and methods, and for results, under each section. Center headings are used for main sections and italicized run-in headings for subsections, and headings should be restricted to these two types only.

Tables. Data should be arranged to facilitate the comparisons readers must make. Tables should be kept small by breaking up large ones if this is feasible. Only about eight columns of tabular matter can be printed across the page. Authors should omit all unessential data such as laboratory numbers, columns of data that

show no significant variation, and any data not discussed in the text. A text reference can frequently be substituted for columns containing only a few data. The number of significant figures should be minimized. Box and side headings should be kept short by abbreviating freely; unorthodox abbreviations may be explained in footnotes, but unnecessary footnotes should be avoided. Leader tables without a number, main heading, or ruled lines are often usful for small groups of data.

Tables should be typed on separate pages at the end of the manuscript, and their position should be indicated to the printer by typing "(TABLE I)" in the appropriate place between lines of the text. (Figures are treated in the same way.)

Figures. If possible, all line drawings should be made by a competent draftsman. Traditional layouts should be followed: the horizontal axis should be used for the independent variable; curves should be drawn heaviest, axes or frame intermediate, and the grid lines lightest; and experimental points should be shown. Labels are preferable to legends. Authors should avoid identification in cut-lines to be printed below the figure, especially if symbols are used that cannot readily be set in type.

All drawings should be made about two to three times eventual reduced size with India ink on white paper, tracing linen, or *blue*-lined graph paper; with any other color, the unsightly mass of small grid lines is reproduced in the cut. Lettering should be done with a guide using India ink; and letters should be 1/16 to 1/8 inch high after reduction.

For difficult photographs, a professional should be hired or aid obtained from a good amateur. The subject should be lighted to show details. A bright print with considerable contrast reproduces best, and all prints should be made on glossy paper.

All original figures should be submitted with one set of photographic reproductions for reviewers, and each item should be identified by lightly writing number, author, and title on the back. Cut-lines (legends) should be typed on a separate sheet at the end of the manuscript. "Preparation of Illustrations and Tables" (*Trans. Am. Assoc. Cereal Chem.* 3: 69–104. 1945) amplifies these notes.

Text. Clarity and conciseness are the prime essentials of a good scientific style. Proper grouping of related information and thoughts within paragraphs, selection of logical sequences for paragraphs and for sentences within paragraphs, and a skillful use of headings and topic sentences are the greatest aids to clarity. Clear phrasing is simplified by writing short sentences, using direct statements and active verbs, and preferring the concrete to the abstract, the specific to the general, and the definite to the vague. Trite circumlocutions and useless modifiers are the main causes of verbosity; they should be removed by repeated editing of drafts.

Editorial Style. A.A.C.C. publications are edited in accordance with *A Manual of Style*, University of Chicago Press, and *Webster's Dictionary*. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities. (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10° C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

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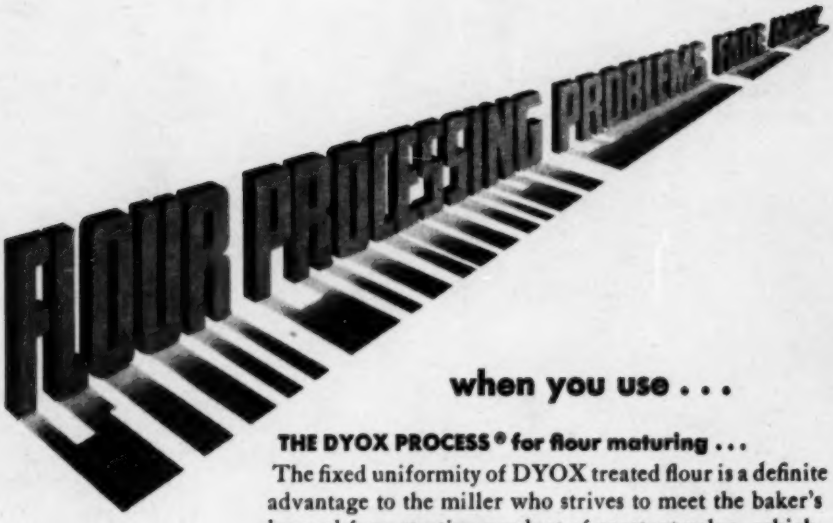

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